# IMPACT OF LIFESTYLE FACTORS ON VASCULAR AGING

by

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Preventing, delaying and managing morbidities is critical to sustain a strong economy and health care system as the proportion of adults over 65 years increases. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in Americans. Signs of CVD are apparent in the vasculature before symptoms of clinical disease are present. Subclinical disease can be measured using non-invasive B-mode ultrasound to assess carotid-femoral pulse wave velocity, common carotid artery intima media thickness, adventitial diameter and lumen diameter. Vascular aging is a natural process that can be modified by lifestyle and pharmacologic interventions. The purpose of this dissertation is to evaluate the impact of lifestyle factors on vascular aging.

Hormone therapy use in early postmenopausal women was associated with significantly smaller adventitial and lumen diameter than those who did not use hormone therapy after adjustment for traditional CVD risk factors. These results concur with findings from another study in which higher levels of endogenous estrogen was related to smaller adventitial diameters. Together, these results

suggest endogenous and exogenous estrogen have similar effects on adventitial diameter.

Sedentary, overweight and obese male young adults at low risk of sleep apnea, and non-snorers had better vascular health, reflected in smaller adventitial and lumen diameters, than those at high risk for sleep apnea and snorers in a cross-sectional study. Results were robust after adjustment for age and systolic blood pressure and add to the literature linking CVD with sleep disordered breathing.

Sedentary older adults who participated in a 6-month physical activity intervention had a clinically relevant decline in arterial stiffness, but greater increases in adventitial and lumen diameters compared to control participants. These findings support that it is never too late to gain cardiovascular benefit from physical activity. Research is needed to understand the implications of arterial diameter following introduction of physical activity to sedentary older adults.

Together these studies support the public health importance of lifestyle factors contribution to subclinical CVD. Each study also provides evidence that measurement of adventitial diameter is critical for a comprehensive understanding of the vascular remodeling processes that accompany these lifestyle changes across the vascular aging continuum.

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# 1.0 DISSERTATION OVERVIEW AND OBJECTIVE

The purpose of this dissertation is to evaluate the effect of lifestyle factors on the vascular aging process measured by B-mode ultrasound in three cohorts spanning the life course. The following questions are addressed in the three papers that are included in this dissertation:

1. The cardiovascular benefit of hormone therapy in postmenopausal women has been controversial. Studies of cardiovascular events and subclinical cardiovascular disease have provided information to better understand that benefit may be limited to women with certain vascular characteristics. The purpose of paper one was to determine if there were differences in adventitial diameter in postmenopausal women currently taking hormone therapy compared to those not currently taking hormone therapy in the Women on the Move through Activity and Nutrition study.

2. Sleep related breathing disorders have been linked to cardiovascular disease and its risk factors. The majority of research on sleep apnea has

been in children, adolescents, middle and older adults; few studies have assessed young adults between 20 and 45 years. The goal of paper two was to determine whether sleep quality and sleep apnea risk were associated with aortic pulse wave velocity, common carotid artery intima media thickness, adventitial diameter and lumen diameter in young adults at the baseline assessment of the Slow Adverse Vascular Effects of obesity (SAVE) randomized trial.

3. Physical activity has been shown to be beneficial to the vasculature of young and middle-aged adults. Studies evaluating the benefits of physical activity on subclinical cardiovascular disease measures in older adults $\geq$ 70 years old have been limited. The objective of paper three was to determine if progression of aortic stiffness, measured by pulse wave velocity, and arterial changes measured by common carotid artery intima media thickness, adventitial diameter and lumen diameter differ in sedentary older adults after a 6-month moderate physical activity intervention compared to an education-only control group in an ancillary study of the Lifestyle Interventions and Independence for Elders pilot trial.

## 2.0 GENERAL INTRODUCTION

"Old age is not a phase of decline and loss, but instead, if approached properly, harbours the opportunity for positive change and productive functioning." – Cicero[1]

### 2.1 PUBLIC HEALTH IMPACT OF THE AGING POPULATION

The American older adult population (defined as $\geq$ 65 years old) is expected to grow from 36.5 million in 2002 to 71.5 million by 2030; this new demographic will make up ~20% of the United States population[2]. Declining birth rates, longer life expectancy and the aging baby boomer generation will all contribute to the changing population distribution[3]. Health care costs which accounted for 16% of the United States Gross Domestic Product in 2008 are also expected to grow[4].

Thus, prevention or delay of chronic diseases like stroke, the leading cause of long-term disability[5], and maintenance of independent living for as long as possible will be even more important for the country's economic resources.

## 2.2 DEFINITIONS OF AGING

Aging is a natural phenomenon beginning at conception and continuing as the organism matures until death. There are different ways to classify age in humans: chronological, biological, societal and subjectively as successful or unsuccessful[6]. Chronological age is the most commonly used definition referring to the amount of time since birth[6]. This definition presumes that the rate of aging in all humans is identical[7]. Chronological age has been a long standing traditional risk factor for cardiovascular disease. Biological age is the presence or absence of pathological processes[6]. Unlike chronological aging, biological aging is unique to each individual and is influenced by evolution and genetics, natural selection[7], and environmental exposures. It has been recommended that age be defined based on normal aging that is universal, degenerative, progressive and intrinsic[6]. This definition should differentiate aging from normal processes of growth and development[6]. Societal age is linked to social norms of what is appropriate for people of different chronological age[6]. Successful aging is a concept captured in Cicero's quote; it is characterized by prevention or delay of chronic disease, morbidity and disability[6]. Positive changes and productive functioning can be the ability to learn new skills and use new technologies that can counteract declines in some activities of daily living. They can also be practicing preventative measures (i.e. eating a balanced diet, participating in physical activity) to delay or slow the progression of disease.

## 2.3 THEORIES OF AGING

The rate of age-associated changes varies based upon many factors including those that are environmental, genetic and a blend of the two[6]. There are several theories of how and why aging occurs that take these factors into account[6, 8]. Two longstanding theories with support from animal and human studies are the rate-of-living hypothesis and free radical theory[9]. They are now combined to explain the complex process of aging. The rate-of-living hypothesis is based upon the premise that an organism's metabolic rate is linked to its life expectancy. This theory was based on observations that most organisms with a higher metabolic rate had shorter life spans than those with lower metabolic rates[10, 11]. Exceptions to this are observed in primates and birds who live considerably longer than predicted[10, 11]. The free radical theory of aging states that chronic oxidative stress in an organism yields oxidative end products[10, 12] also known as reactive oxygen species (ROS) that cause cellular damage to the body[13]. ROS effect biological tissues in the same way although the response to cumulative damage is different based on the type of cell[7]. ROS are known to accelerate telomere attrition leading to accelerated cell senescence. Senescence occurs when a cell can no longer replicate[14]. The combination of the rate-of-living and free radical theories postulates that a higher metabolic rate produces more ROS; thus, contributing to a shorter life span[10].

## 2.4 TELOMERE AND AGING

Like the caps on the end of shoe laces, telomere are specialized DNA-protein complexes at the end of eukaryotic chromosomes[15] that preserve genome integrity and stability[16, 17]. Telomere shorten with each cell division in somatic cells[18]. As a result, studies have shown biologically older organisms have shorter telomere than younger ones in animal models[11] and in humans[19]. The rate of telomere shortening is influenced by genetic and environmental factors[20]. Telomere length has X-linked inheritance[21]. Consequently, telomere length is largely heterogeneous between individuals, and cells types within individuals[19]. Although there are no differences in telomere length in newborn males and females[22] in adulthood females have longer telomere than age-matched males [21, 23-27] which may be a result of slower biological aging in females. These differences may also be related to estrogen that will be further discussed in Chapter 3. Individuals with premature/accelerated aging syndromes (i.e. Werner syndrome, ataxia telangectasia, dyskeratosis congenita) show accelerated telomere shortening compared to individuals without these conditions[28]. Cross-sectional and cell culture studies have shown some traditional cardiovascular risk factors are associated with shorter telomere length including hypertension[29], diabetes[30], dyslipidemia, homocysteine[31, 32], obesity[33-36], smoking[33, 35], and response to stress[37]. Cross-sectional studies of individuals with a greater burden of subclinical cardiovascular disease[27, 33], those with

cardiovascular disease[38, 39], and those with cerebrovascular disease[40] also have shorter telomeres. These findings support evidence that cardiovascular risk factors and disease reflect accelerated vascular aging.

## 2.5 VASCULAR AGING

Vascular aging, or age-associated changes that occur throughout the blood vessels of the arterial tree, is characterized by many physiological changes. These changes affect genetic and functional responses in the body. The rate of vascular aging is likely influenced by underlying physiological alterations resulting from arterial pulse pressure widening, hypertension, dyslipidemia, diabetes, genetic factors[41], inactivity, and obesity. Some of these vascular age-related changes are reduced compliance, increased inflammatory response[42], increase in vascular smooth muscle cells, impaired angiogenesis[43, 44], reduced anti-thrombogenic property of the endothelium[45], thickening of the intima media, dilation of the lumen [46] and increased adventitial diameter.

Atherosclerosis is a disease of the arteries that begins as a fatty cholesterol streak in the inner wall of the artery; regression or further development is dependent upon several factors. Atherosclerosis in young adults may reflect accelerated aging of the vascular wall[41, 47] indications of this process are arterial stiffening and increased adventitial diameter.

### 2.5.1 Arterial Anatomy

The arteries are composed of three layers: the adventitia, the media and the intima. The media layer is sandwiched between the external elastic lamina at the adventitia-media boundary and the internal elastic lamina at the media-intima boundary[48]. Each arterial layer has a distinct purpose, thus is composed of different cell types with varying densities. The adventitia is the outermost layer of the artery; it is composed of mainly fibroblasts, collagen, and elastin fibers aligned longitudinally; mast cells, macrophages and ganglion cells can also be found in the adventitia[48]. Its composition provides structure to the artery and is critical for homeostasis of the vessel wall[49]. The media, the middle layer of the artery, is made of layers of smooth muscle cells[48] that provide stability and resiliency[50]. The intima is the inner most layer of the artery; it is a single layer of intertwined endothelial cells[48] attached to a thin extracellular matrix[49]. The main purpose of the endothelium in arteries is to separate the circulating blood compartment from the tissue of the vascular wall[49].

### 2.5.2 Shear and Tensile Stress

The blood vessel adapts to environmental changes through structural and functional changes. This adaptation is driven by two hemodynamic processes: shear and tensile stress[50]. Shear stress is the tangential, mechanical force exerted on the vessel from the flow of blood; tensile stress is the force

perpendicular to the wall of the artery pushing outward on the inner artery wall[50]. Shear stress is affected by changes in blood pressure and tensile stress is affected by changes in wall tension and wall thickness. Optimal homeostatic conditions are met at a specific, constant level of shear and tensile stress. Many of the vascular changes associated with aging—vascular stiffening, intima media thickening and enlarged adventitial diameter—disrupt the balance of shear and tensile stress.

### 2.5.3 Collagen & Elastin

Collagen and elastin are very important components of the arterial wall. They are basic components of the adventitia, the outermost layer of the blood vessel. Collagen fibrils provide structure to the artery wall matrix, bearing the majority of tensile stress, and preventing excess distension[51]. The elastin network provides tensile stress support, elasticity, resiliency, and allows the artery to respond to stress exerted by hemodynamic conditions by distributing stress evenly throughout the wall by dilation, constriction and absorption[50]. The quality[54] of elastin in the artery walls declines with age[52] as a result of inadequate production and increased fragmentation and fracture[46]. The increased collagen content interacts with free amino groups and forms advanced glycation end products that increase cross-linking[46]. Increased cross-linking of

collagen and decreased quality and function of elastin causes increased arterial stiffness reflected by higher pulse wave velocity[53].

### 2.5.4 Intima Media Thickening

The intima and media thicken with age. These changes are reflected in intima media thickness(IMT) which is strongly correlated with intrinsic arterial aging[41]. Several studies have shown thicker IMT in older participants compared to younger ones. There is a doubling or tripling of IMT in people between the ages of 20 and 90[54]. Rodent[55] and nonhuman primate[56] models of aging reflect a thicker intima in older animals than in younger ones. The animal models both observe intima medial thickening in the absence of atherosclerosis[55, 56] suggesting that intima medial thickening is part of the normal aging process. Normal thickening is characterized by smooth muscle cell hypertrophy[57].

### 2.5.5 Blood Pressure Changes

The vascular system or arterial tree has two essential functions: 1. deliver blood from the left ventricle to capillaries of the organs and tissues that need it, and 2. cushion the pulsatile energy from the blood flow so that it is continuous[58, 59]. Aging vasculature is associated with reduced compliance, distensibility[60, 61], baroreflex response[60, 62], cardiac output while supine at rest[60, 63, 64], and increased sensitivity to salt or sodium[65]; these factors all contribute to changes in

blood pressure. Blood pressure has two components: a pulsatile component represented as systolic blood pressure (SBP) and a steady state component represented as diastolic blood pressure (DBP). Arterial pressure is a balance between peripheral resistance, known to increase SBP & DBP, and central artery stiffness, known to increase SBP and lower DBP[41]. In young adults blood pressure is driven more by peripheral resistance, while blood pressure in older adults is driven by central artery stiffness explaining the increase in SBP and decrease in DBP observed in older adults (isolated systolic hypertension) and the increase in both in younger adults[58, 66].

Isolated systolic hypertension is elevation of brachial systolic blood pressure when diastolic blood pressure is normal or below normal and pulse pressure is elevated[67]. Isolated systolic hypertension is most prevalent in adults over 60 years of age[67, 68]; it effects 15% of adults over 60[69]. Arterial stiffening is the main contributing factor of isolated systolic hypertension[67, 68, 70]. This stiffening is directly related to changes in elastin, collagen, and nitric oxide production. Other contributing factors are increased stroke volume, diminished capacity of the aortic valve and arteriovenous shunts[67]. Increased mortality and stroke were associated with isolated systolic hypertension in a meta analysis of 8 trials of isolated systolic hypertension patients $\geq$60 years old(n=15693)[71]. Treatment of isolated systolic hypertension lowered total mortality by 13%, cardiovascular mortality by 18%, cardiovascular complications by 26% and stroke by 30%[71].

Obesity, smoking and low educational attainment appear to contribute to the phenomenon of isolated systolic hypertension in young adults under 40 years old from the National Health and Nutrition Examination Survey[72].

### 2.5.6 Reactive Oxygen Species (ROS) and Oxidative Stress

Reactive Oxygen Species (ROS) are known to accelerate telomere attrition in vascular smooth muscle cells[31, 73] leading to accelerated senescence. The senescence of vascular endothelial cells may lead to endothelial dysfunction especially in areas susceptible to or with already developed atherosclerotic damage[74]. Oxidative stress is a physiological state when there are more ROS than can be broken down by the cellular environment[75, 76]

### 2.5.7 Nitric Oxide Production Changes

Nitric oxide (NO) plays a critical role in the vascular system, especially in the endothelium. NO is a potent vasodilator, produced in the endothelium as the response to antagonists and shear stress[77]. NO can act as both an antioxidant and a pro-oxidant. Its role is determined by the chemical[78] and physiological environment[79]. As an antioxidant it is necessary for providing protection against leukocyte adhesion, thrombocyte aggregation, and smooth muscle cell proliferation[80]. Vessels in older organisms in different vascular beds in animal and human models[81] show reduced response to NO-dependent vasodilator

response to acetylcholine[82], and when hyperlipidemia, hypertension, or diabetes is present[79]. These risk factors also slow NO synthesis and quicken destruction of the molecule[77]. Aging is associated with diminished sensitivity to $\beta_2$-adrenoceptor agonists leading to impaired vasoconstrictor response[83].

## 2.6 SUBCLINICAL CARDIOVASCULAR DISEASE MEASURES

Subclinical cardiovascular disease, early signs of cardiovascular disease before symptoms or an event, can be measured using non-invasive, B-mode ultrasonography. These measures are used in this dissertation and are described below.

### 2.6.1 Common Carotid Artery Adventitial Diameter

Adventitial diameter (AD), also referred to as inter-adventitial diameter in the literature, is a non-invasive measure of vascular health. AD can be measured at different arterial sites; this document will refer to AD measured in the common carotid artery unless otherwise specified. It is defined as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interfaces on the far wall. AD has been overlooked as a measure of interest because it was thought that lumen diameter was the measure that provided the most important information about vascular remodeling[48]. This is true for the coronary

arteries but not for carotid arteries, the focus of this dissertation. AD is reproducible and reliable[84, 85]. AD is associated with several traditional cardiovascular risk factors and cardiovascular disease. Larger adventitial diameters are associated with aging[86-88], male gender[89, 90], higher blood pressure[84, 89, 90], glucose[90], insulin, waist circumference, body mass index[85, 86], lipids[90], increased atherosclerotic lesions[91] and plaques[88], pre-existing coronary heart disease[86, 92, 93] and prevalent cardiovascular disease [92-95].

### 2.6.2 Common Carotid Artery Lumen Diameter

Lumen, luminal or inner diameter is a non-invasive measure of the artery often paired with and strongly correlated with intima-media thickness. It is a reproducible and reliable measure[84, 96]. Lumen diameter (LD) is larger in men than in women[89, 90, 96-98] and is positively correlated with older age[97], higher SBP[96], mean arterial pressure[98], body mass index (BMI)[96, 98], cholesterol, apo-B[96], pre-existing coronary disease[92] and intima media thickness.

### 2.6.3 Common Carotid Artery Intima Media Thickness

Intima media thickness (IMT) is one of the most commonly used subclinical B-mode ultrasound measures used. It is measured as the distance between the lumen-intimal interface and the medial-adventitial interface. The measurement varies depending on whether both the left and right side, or one side is imaged,

the segment(s) of the artery used (common carotid, internal carotid, bulb), whether near and far wall images are used, and if plaque is included in the measurement. IMT is typically the average of all segments and sides available. It is thicker in men than age matched women[90]. IMT is reliable, reproducible[99] and measured in a wide variety of populations in several large epidemiological studies[89, 100-105]. These studies have demonstrated the relationship between thicker IMT and traditional cardiovascular risk factors like age[104], smoking[100, 103, 104], hypertension[100], higher low density lipoprotein (LDL) cholesterol [100, 104] ; they have also shown that thicker IMT predicts pre-existing coronary heart disease[92], coronary heart disease[101], stroke[102], and myocardial infarction[102, 105].

### 2.6.4 Carotid Plaque

Carotid plaque is an indicator of atherosclerosis and predictor of myocardial infarction[106, 107] and cardiovascular mortality[107]. Carotid plaque can be assessed using B-mode ultrasound imaging. In this dissertation it is defined as a distinct area protruding into the vessel lumen that is at least 50% thicker than the adjacent IMT. The degree of plaque is graded between 0 and 3 for each segment. A grade of zero is defined as no visible plaque. A grade of one was defined as one small plaque that is less than 30% of the vessel diameter. A grade of two is defined as one medium plaque that is 30-50% of the vessel diameter or multiple small plaques. A grade of 3 is defined as a large plaque

that is greater than 50% of the vessel diameter or multiple plaques with at least one medium plaque. The plaque variable used was dichotomized as no plaque or any plaque.

### 2.6.5 Carotid-Femoral Pulse Wave Velocity

Increased pulse wave velocity (PWV) with age is observed even in populations with little or no atherosclerosis[108]. Therefore, stiffening is not a definite indicator of atherosclerosis in all situations. It can be a characteristic of natural aging. Carotid-femoral PWV (cf-PWV), also known as central or aortic PWV, is a reproducible and reliable[109, 110], non-invasive measure of arterial stiffness. The pulsatile flow of blood creates a pressure waveform that carries energy from the heart throughout the arterial tree is reflected. Cf-PWV is calculated as the distance the pulse wave travels from the carotid artery to the femoral artery divided by the time it takes the wave to travel this distance. A higher PWV reflects a stiffer vessel and increased cardiovascular risk factors and events than a lower PWV. Higher cf-PWV is associated with older age[104, 111, 112], sedentary lifestyle[112], higher blood pressure[113], central adiposity[114], diabetes[115], and is a predictor of hypertension[113], coronary heart disease[116-118], stroke[116, 118], cardiovascular mortality[115, 117-120] and all-cause mortality[115, 120].

## 2.7 LIFESTYLE FACTORS

### 2.7.1 Sleep and Obstructive Sleep Apnea

Sleep is essential for health, wellbeing and the ability to function at our best. It is not a passive activity; during sleep the body repairs and regenerates tissues, builds bone and muscle, and secretes hormones. This is accomplished during two sleep cycles, rapid eye movement (REM) and non-rapid eye movement (non-REM), and 5 stages of sleep[121]. Approximately 50% of sleep in children is REM sleep[122],[122] compared to 20% in adults and declines with age[121]. The average adult needs 7-8 hours of sleep daily; < 6 hours of sleep or > 8 hours of sleep have been indicative of higher risk of all-cause mortality[123]. Data from two cohort studies of men and women from the United States and the United Kingdom found that short sleep duration (<6 hours) was correlated to obesity and poor health, while long sleep duration (>8 hours) was associated with comorbidities[124].

Sleep apnea (SA) is disordered breathing caused by repeated episodes of reduced air flow (hypopnea) and complete cessation of air flow (apnea) caused by collapse of the pharyngeal airway during sleep. The severity of SA is determined by the number of apneas and hypopneas per hour of sleep or the

Apnea Hypopnea Index (AHI)[125-127]. Mild SA is defined as an AHI of 5-14, moderate an AHI 15-29 and severe an AHI 30 or greater[126].

Obstructive Sleep Apnea (OSA) is the most common type of SA with an estimated prevalence of 24% in men and 9% in women 30-60 years old in the United States[128] but is usually undiagnosed[126, 129]. OSA is most strongly linked to obesity[130, 131], male gender[132] and age[127, 132]. It is also associated with hypertension[133, 134], diabetes and insulin resistance [134, 135], higher PWV[136] and IMT[136, 137] compared to controls and less severe OSA participants. There is a great deal of literature on OSA in children and middle and older adults but there is gap in the literature on OSA in young adults 18-30 years old[138]. Possible reasons could be OSA is not thought to be a disease of young adults because they often do not report symptoms. Laboratory polysomnography is the gold standard for sleep evaluation and diagnosis of SA although its use in epidemiological studies are limited due to the time-consumption of the test, participant burden, and expense of the equipment and reading the data[139]. As a result portable home monitors[140] and questionnaires[141] have been developed as effective measures to determine those at risk for SA.

### 2.7.2 Physical Activity in Older Adults

Physical activity elicits beneficial changes in the cardiovascular system and in some cardiovascular risk factors. Results of intervention studies show increased physical activity lowers CV risk[142-145]. Brisk walking has been shown to reduce the risk of CV events[144], blood pressure[143], myocardial infarction[145] and coronary heart disease[142]. Physical activity is thought to slow the progression of atherosclerosis by effecting weight, blood pressure, insulin sensitivity, glycemic control, lipid profile, fibrinolysis, endothelial function and inflammatory response systems[146]. These changes slow the deterioration of function and health observed with aging, even in older adults in observational studies and short duration clinical trials [147]. However, the duration and intensity of physical activity required to produce clinically meaningful improvements in the vasculature of older adults is unknown.

Compliance reflects how well and artery responds to changes in pressure and volume by expanding and recoiling. Compliance is reduced with age as arteries lose their elasticity and volume change is reduced. Aerobic activity improves arterial compliance in middle-aged and older adults[148-150] without isolated systolic hypertension[151].

To date, the evidence of physical activity's effect on arterial stiffness, measure by PWV is inconclusive. Two cross-sectional studies of physically active endurance trained older men from the Baltimore Longitudinal Study of Aging[150] and endurance trained postmenopausal women[152] found the endurance trained individuals had less arterial stiffness, measured by PWV, than their sedentary age-matched peers. However, two randomized studies of adults with elevated blood pressure (an eight week cross-over cycling or sedentary lifestyle intervention in men,[151] and a three month walking intervention versus sodium restriction control group in postmenopausal women[153]) did not find differences in change in arterial stiffness, also measured by PWV, between active and sedentary groups[151, 153]. The short duration of the interventions ($\leq$ 3 months), the study populations and the comparison group in the case of the latter study, may explain the findings. High blood pressure may slow the positive effects of physical activity[151, 153], thus requiring a longer duration of activity before benefit is observed. Additionally, the postmenopausal women in the sodium restriction control group had a large reduction in arterial stiffness[152]. Consequently, the effect of physical activity was not compared to sedentary behavior alone.

The evidence of physical activity's effect on intima media thickness is also inconclusive. A cross-sectional study of physical activity level assessed by questionnaire did not find a significant trend in IMT across the three groups of activity level in a cohort of men and women 65 and older[154]. Another cross-

sectional study that compared sedentary and endurance trained adults also found no relationship between activity and IMT[154, 155]. A third cross-sectional study found that active adults who ate a healthy diet had lower IMT than sedentary adults who ate an unhealthy diet[156]. However, this relationship was only present in non smokers; diet and activity were not examined separately in this study[156]. Finally, a longitudinal study observed slower progression rates of IMT in active peri- and postmenopausal women compared to the sedentary age-matched peers[157].

To better understand the effects of physical activity on the vasculature of older adults a randomized physical activity intervention of longer than three months is needed.

# 3.0 THE EFFECTS OF HORMONE THERAPY ON COMMON CAROTID ARTERY ADVENTITIAL DIAMETER IN EARLY POSTMENOPAUSAL WOMEN

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## 3.1 INTRODUCTION

Observational studies of hormone therapy (HT) have consistently suggested a cardiovascular (CV) benefit of estrogen[1], while clinical trials have shown an increase in CV events among women randomized to HT use[2, 3]. Recent animal data, as well as observational and clinical studies suggest that in postmenopausal women, the effects of estrogen on the vasculature may vary by age, years since menopause, degree of atherosclerosis at time of therapy initiation[4-8], method of administration and composition (estrogen versus estrogen and progesterone). Possible benefits of HT on subclinical CV disease have been observed in randomized studies of younger women evaluating coronary artery calcium[9] and common carotid artery (CCA) intima media thickness (IMT)[10, 11]. Postmenopausal women, aged 50-59 years, who had undergone hysterectomy and were on estrogen only HT had 30-40% less coronary artery calcium than those randomized to placebo; these results were robust in adjusted and unadjusted models[9]. Long term ($\geq$ 2 years) HT use in postmenopausal women diminished age associated changes in IMT; short-term use and non-use did not[10, 11].

Despite wide use of HT, the direct effect of hormones (endogenous or exogenous) on the vasculature in humans is not completely understood. Recent

data from the Study of Women's Health Across the Nation (SWAN) suggest that declining endogenous estrogen with the menopausal transition has a direct effect on the peripheral vasculature[12]. Lower levels of estradiol were significantly associated with larger CCA adventitial diameters (AD) even after adjustment for CV risk factors[12].

Larger adventitial diameters are associated with aging[13, 14], CV risk factors [12, 15-17], and prevalent cardiovascular disease[18-21]. Thus, the increase in AD observed with declining endogenous estrogen[12] suggests that lower levels of endogenous estrogens are associated with a less healthy vasculature. The strong association between AD and endogenous estrogen suggests a similar association may exist with the use of exogenous estrogen. The purpose of this study is to determine whether early postmenopausal HT users have significantly different AD than those who were not current users of HT in the Women on the Move through Activity and Nutrition (WOMAN).

## 3.2 METHODS

### 3.2.1 Study Population

The WOMAN study is a randomized clinical trial testing the ability of non-pharmacological lifestyle intervention to modify CV risk factors in postmenopausal women[22]. The study recruited 508 eligible African American and Caucasian women from Allegheny County, Pennsylvania between April 2002 and October 2003 through direct mailings. Briefly, eligible women were postmenopausal, between 52 and 62 years of age, able to walk, currently using HT, willing to participate in either intervention group regardless of assignment and had a waist circumference of $\geq$80 cm, a body mass index (BMI) between 25.0 and 39.9 kg/m$^2$, blood pressure < 160/95 mmHg, and low density lipoprotein (LDL) cholesterol between 100 and 160 mg/dL. Women were ineligible if they were taking medication for cholesterol, diagnosed with or on medication for diabetes, diagnosed with a psychotic disorder, or suffering from depression[22]. Written informed consent was obtained from all the participants. The study was approved by the University of Pittsburgh's Institutional Review Board. The results of the WHI estrogen/progestin arm were published in the middle of recruitment[3]; as a result the eligibility criterion of current use of HT was modified to current or recent history of hormone use[22]. Recent history of hormone use was defined as

prior use of at least 2 years within 6 months of randomization. The decision to remain on HT was determined by the participant and her physician. At baseline, 40% of the women had discontinued use of HT (these women will be referred to as HT non users) and 60% remained on HT (these women will be referred to as HT users). The effects of HT diminish quickly after use is discontinued. The primary endpoints of the study were coronary artery calcium, pulse wave velocity and CCA IMT.

### 3.2.2 Carotid Ultrasound Measures

CCA IMT, AD, lumen diameter (LD) and plaque were assessed by B-mode ultrasound using the Toshiba SSA-270A duplex scanner (Toshiba American Medical Systems, Tustin, CA) with a 5-MHz linear array transducer. Right and left carotid images were taken of the near and far walls of the distal common carotid artery 1 cm proximal to the carotid bulb[22]. IMT was defined as the distance from the lumen-intimal interface to the medial-adventitial interface. AD was defined as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall. LD was defined as the distance from the intima-lumen interface of the near wall to the lumen-intima interface of the far wall. The interfaces were traced electronically over the distal CCA and a computer generated measurement was obtained for

each pixel in the area of interest; these measurements were averaged to determine the IMT, AD, LD used for this analysis.

Plaque was determined based on grading each of the 5 segments of the left and right carotid artery (distal and proximal CCA, carotid bulb, and proximal internal and external carotid artery). Plaque was defined as a distinct area protruding into the vessel lumen that was at least 50% thicker than the adjacent IMT. For each segment the degree of plaque was graded between 0 and 3. A grade of zero was defined as no visible plaque. A grade of one was defined as one small plaque that was less than 30% of the vessel diameter. A grade of two was defined as one medium plaque that was 30-50% of the vessel diameter or multiple small plaques. A grade of 3 was defined as a large plaque that was greater than 50% of the vessel diameter or multiple plaques with at least one medium plaque. Plaque was dichotomized into no plaque or any plaque.

### 3.2.3 Visits

The first of two pre-randomization screening visits included a 12-hour fasting blood draw, physical measures of height, weight, waist circumference, blood pressure, the long distance corridor walk, medical, physical activity and weight history. Conventional enzymatic methods were used to obtain total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride concentrations from

the blood samples[22]. LDL cholesterol was estimated using the Friedewald equation[22]. Medical history included history of drug, vitamin/mineral supplement, and alcohol use[22]. CCA AD, LD, IMT and plaque were measured at the second screening visit[22].

### 3.2.4 Analytical Methods

Five hundred eight women were randomized into the WOMAN study. Seventeen women had incomplete data for the calculation of AD or IMT and were excluded from this analysis leaving a final sample size of 491 women. All analyses were completed using SAS v9.1 (SAS Institute Inc., Cary, NC). A p-value $< 0.05$ was considered statistically significant.

Descriptive statistics and normality of continuous measures were assessed for the cohort. Differences in demographic, anthropometric and health characteristics between the users and non users were determined using chi-square analyses for categorical variables and either t-tests or Wilcoxon-rank sum tests for continuous variables.

Simple linear regression was used to assess univariate associations between AD and HT use and AD with the following CV risk factors: age, race, systolic and diastolic blood pressure, pulse pressure, BMI, weight, height, waist

circumference, total, LDL and HDL cholesterol, triglycerides, glucose, insulin, smoking and antihypertensive medication use. Multivariable linear regression was used to test for the following predetermined covariates: age, race, pulse pressure, and smoking status and any significant variable in the univariate analysis. The predetermined covariates were selected based on their association with AD in other studies. When collinearity was suspected($r > 0.4$), Spearman correlations between the variables were determined and the variable most strongly correlated to AD was selected for the analysis. Pulse pressure was chosen over systolic blood pressure, weight over waist circumference and BMI, and glucose over insulin for the multivariable model. The most parsimonious model was chosen.

## 3.3 RESULTS

The mean age of the women was 57 ± 3.0 years, mean BMI was 41.2 ± 3.8 kg/m$^2$; 11% were African American and 6% were current smokers. There were 197 HT non users and 294 HT users at the time of the baseline carotid ultrasound scan. HT non users were older and had a higher percentage of African Americans. Overall HT non users had a significantly worse CV disease risk profile than HT users: higher total cholesterol, LDL cholesterol, glucose and insulin and lower HDL cholesterol (Table 3.1). There were, however, no statistically significant differences in blood pressure, measures of general or central obesity, and smoking status by HT status (Table 3.1).

The AD and LD were larger in the HT non users than in the users ($p = 0.0013$, $p = 0.0024$, respectively, Table 3.2). However, IMT and presence of plaque were similar between the two groups (Table 3.2).

Simple linear regression showed that AD was smaller in HT users, AD was significantly positively associated with SBP, pulse pressure, BMI, weight, height, waist circumference (all $p <0.001$), glucose, insulin (all $p=0.01$) and use of antihypertensive medications, and negatively associated with African American race, and cigarette smoking and ($p<0.05$) (Table 3.3). HT use, African American

race and cigarette smoking were associated with smaller AD. All of the other risk factors were positively associated with AD.

The final multivariate model forced age, race, pulse pressure, and smoking status as covariates (Table 3.4). The model was also run controlling for anti-hypertensive medication use, but this variable fell out of the multivariable model when pulse pressure was added. HT non-use, higher pulse pressure, and higher weight were significantly associated with larger AD (all $p < 0.01$, Table 3.4). HT use was associated with a 0.16 mm smaller AD. Current cigarette smokers had 0.18 mm smaller AD than current non-smokers, with borderline significance (Table 3.4). Age, race and smoking status were not statistically significant in the final model.

## 3.4 DISCUSSION

Early postmenopausal HT users had significantly smaller AD than HT non users. This relationship remained after adjustment for known CV risk factors. The HT users also had significantly smaller LD than the non users. This is clear evidence that HT is associated with vascular geometry in early postmenopausal women.

The current study found that exogenous estrogen has similar associations with AD as endogenous estrogen as seen in the SWAN Heart study[12]; smaller AD was associated with higher levels of estrogen. LD was also significantly smaller in the HT group in the current study. Similar results were found in this study in a cross-sectional study of non-oral (percutaneous gel or transdermal patch) HT. HT users had smaller LD than non-users[11] which is consistent with these findings. Together, these findings suggest the positive effect of estrogen on the vasculature may be through maintenance of vascular structure and function. The adventitia, the outer most layer of the artery, is composed of supportive connective tissue, fibroblasts, collagen, and elastin fibers[23]. Estrogen is known to preserve arterial structure by slowing elastin and connective tissue degradation, and slowing age- and estrogen-related increases in collagen that increase vascular stiffening[24]. A small diameter reflects a healthy vasculature that is able to maintain an optimal balance of shear and tensile stress[25-27]. An enlarged

diameter is unable to effectively control levels of shear stress. This can make the artery vulnerable to injury and atherosclerotic development[17, 23]. The current study and the SWAN Heart study found that larger AD was associated with older age, higher systolic blood pressure, higher glucose and higher insulin: all risk factors for CVD. Additional supporting evidence that enlarged diameter is an indicator of poor vascular health come from several studies showing enlarged AD is associated with CV disease risk factors[12, 13, 17], increased IMT[20], plaque[20, 28], and acute coronary events[20].

Arterial diameter enlargement precedes intimal thickening and may explain why arterial diameter differences in HT users and non users were observed in this study but differences in IMT were not. These findings contribute to the mixed findings of prior studies of HT and IMT[10, 11, 19, 29-33]. Prior studies have been of American[19, 30], Australian[32], French[11, 31], German[29], and Japanese[10, 33] women, undergoing diverse types of HT regimens. The variability in study design complicates the ability to draw conclusions. There is evidence that the human response to ovary-produced 17β-estardiol and estrone is different than the response to equine derived conjugated equine estrogen[34]. This is due to equine estrogens that are not found in humans and their affinity to the estrogen receptors compared to the other forms of estrogen[34]. In addition, the route of estrogen therapy affects the physiological response of the body. Transdermal therapy bypasses processing through the liver resulting in no adverse increase in

triglyceride[35], or C-reactive protein[36] seen when oral therapy is used. This may explain why both studies of transdermal therapy found IMT and IMT progression to be significantly different in HT users and non HT users[11,33]. HT users had significantly thinner IMT than non-users in the cross-sectional study[11]; HT users had significant reductions in IMT progression after a year compared to non-users in the longitudinal study[33]. Another longitudinal study compared oral and non-oral therapy[31]. Women who used transdermal HT had statistically significant greater IMT than women who used oral HT after four years[31]. Two out of three cross-sectional studies of oral HT use found significantly thinner IMT in HT users[10, 32] although in one study this was only true in the long term ($\geq$ 2 years) HT users[10]. This suggests that the duration of HT use is important for observation of detectable changes in IMT. The Atherosclerosis Risk in Communities (ARIC) study and the current study had several similarities: both were cross-sectional, had American participants who used oral HT, who were relatively younger and had fewer years on HT compared to the positive studies. ARIC found no significant difference in IMT by HT use[19], as the current study did. The ARIC study also had undefined HT regimen because the regimen was predetermined by the woman and her physician prior to the study. Selection bias may be present in both studies since women who chose to go on HT or continue HT may have been different than those who did not. Longitudinal studies of the affect of HT on IMT progression have had mixed results[6, 29-31, 33]. Some have found no differences in IMT progression in HT users and non users[29, 31], others have observed slower

progression in IMT in HT users than in HT non users[6, 30, 33]. The longitudinal study of oral therapy that did not find a difference in IMT progression in HT users and non-users had 1 year of follow-up[29].

Three studies evaluated differences in HT and IMT in women by age[10, 11, 32]. The women were dichotomized into younger vs. older (using 55 or 60 as the age cut-point) significant differences in IMT were observed only in the older women who had longer use of HT than the younger women[10, 11, 32]. This may suggest that the effects of estrogen on IMT are evident after long-term use.

Current smoking was associated with a smaller AD in the current study; this is contrary to the findings of the Atherosclerosis Risk in Communities (ARIC) Limited Access Data study[13] that included men and women. McGarth et al observed smokers that used HT had significantly thinner IMT (0.65±0.01 mm) than HT non-users (0.75±0.01 mm, p=0.002); there was no significant difference observed in non-smokers (0.68±0.01 HT users v 0.70±0.03 non-users, p=0.7)[32]. The McGarth study suggests that these findings may be the result of estrogen's effect on the vasculature of smokers, who are usually discouraged from using HT due to the risk of thrombosis[32]. Other explanations are the vasoconstrictive properties of cigarette smoking[37-39] and the physiologic changes of the composition of the artery wall with age, and the number of pack years of cigarette use.

Strengths of this study are that it fills a gap in the literature because it is one of the first to evaluate AD and HT, the methods used are valid and reliable, the lab that performed the ultrasound measures has high quality control, the study population is well defined. The contribution of the adventitia to vascular function has been largely ignored[23]. This is evident in the scarcity of literature that evaluates the measure. High resolution B-mode ultrasound is a valid and reliable detector of structural atherosclerotic changes of the arterial walls[40]. The ultrasound measures in this study were performed with excellent reproducibility and continuous quality control to ensure the data obtained is reliable and valid. The ultrasound technicians were all certified and recertified every 6 months. The study sample is a well defined population of racially diverse postmenopausal women from the Allegheny County and surrounding areas of western Pennsylvania.

A limitation of this study is that the HT regimen was varied since the dose, hormone composition (estrogen only or estrogen plus progestin) and form were chosen prior to the study by the participant and her health care provider. The main type of HT was oral. One study observed that transdermal HT had greater effects on IMT than oral HT[31]. A standard dose and regimen of HT would be easier to evaluate and compare to previous studies. This would likely be especially true for the IMT results that were not significant in this study. Another limitation is that the adherence to HT and the level of estrogen or estradiol in the

users and non users was not assessed in this study. Although the women on HT reported use we do not know their adherence rates or the amount of estrogen metabolites during the ultrasound measurements. In the future, assessment of estradiol levels should be included for better understanding.

In conclusion, these data suggest that HT use affects the vasculature in the early postmenopausal period. AD is important in assessing vascular changes and should be evaluated with lumen diameter and intima media thickness to provide a more complete story of vascular response and health.

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## 3.7 DISCLOSURES

There are no disclosures.

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## 3.9 TABLES FOR CHAPTER 3

Table 3.1 Baseline demographic, anthropometric and health characteristics of WOMAN cohort by HT use

| | Non Users (n=197) | Users (n=294) | |
|---|---|---|---|
| | mean ± standard deviation or % | | p-value* |
| Age, years | 57.4 ± 2.9 | 56.7 ± 2.9 | 0.006 |
| African American Race, % | 16.9 | 7.2 | 0.008 |
| Systolic Blood Pressure, mmHg | 123.6 ± 13.0 | 123.7 ± 13.4 | 0.899 |
| Diastolic Blood Pressure, mmHg | 76.7 ± 7.6 | 76.1 ± 8.0 | 0.349 |
| Pulse Pressure, mmHg | 46.8 ± 10.5 | 47.7 ± 10.8 | 0.335 |
| Body Mass Index, kg/m$^2$ | 30.7 ± 4.0 | 30.7 ± 3.6 | 0.793 |
| Weight, kg | 81.8 ± 11.6 | 81.4 ± 11.3 | 0.849 |
| Height, cm | 163.3 ± 5.6 | 162.9 ± 5.9 | 0.642 |
| Waist circumference, cm | 105.4 ± 11.8 | 105.9 ± 10.7 | 0.520 |
| Cholesterol, mg/dL | 223.1 ± 29.0 | 212.5 ± 26.7 | 0.0002 |
| High Density Lipoprotein, mg/dL | 58.9 ± 13.7 | 61.4 ± 14.4 | 0.052 |
| Low Density Lipoprotein, mg/dL | 137.1 ± 26.0 | 122.1 ± 22.8 | <0.0001 |
| Triglycerides, mg/dL | 135.6 ± 63.5 | 146.3 ± 80.2 | 0.222 |
| Glucose, mg/dL | 97.3 ± 9.0 | 93.8 ± 9.0 | <0.0001 |
| Insulin, mg/dL | 14.6 ± 7.1 | 13.0 ± 6.4 | 0.004 |
| Current Cigarette Smoker, % | 8.1 | 5.1 | 0.181 |
| Antihypertensive Medications, % | 25.9 | 21.4 | 0.251 |

*chi-square for categorical, t-test for continuous

Table 3.2 Baseline subclinical measures of cardiovascular disease of WOMAN cohort by HT use

| | **Non Users (n=197)** | **Users (n=294)** | |
|---|---|---|---|
| | mean ± standard deviation or % | | p-value* |
| Adventitial Diameter, mm | 6.9 ± 0.54 | 6.8 ± 0.52 | 0.001 |
| Lumen Diameter, mm | 5.4 ± 0.48 | 5.3 ± 0.46 | 0.002 |
| Intima Media Thickness, mm | 0.72 ± 0.10 | 0.72 ± 0.09 | 0.900 |
| Presence of Plaque, % | 30.5 | 29.6 | 0.838 |

*chi-square for categorical, t-test for continuous

Table 3.3 Univariate linear regression showing the association of demographic & cardiovascular risk factors with common carotid artery adventitial diameter

| | Model | | |
|---|---|---|---|
| | β | Standard Error | p-value |
| Hormone Therapy Use | -0.157 | 0.048 | 0.001 |
| Age, years | 0.017 | 0.008 | 0.037 |
| African American Race | -0.187 | 0.076 | 0.014 |
| Systolic Blood Pressure, mmHg | 0.007 | 0.002 | <0.0001 |
| Diastolic Blood Pressure, mmHg | 0.003 | 0.003 | 0.410 |
| Pulse Pressure, mmHg | 0.010 | 0.002 | <0.0001 |
| Body Mass Index, $kg/m^2$ | 0.031 | 0.006 | <0.0001 |
| Weight, kg | 0.013 | 0.002 | <0.0001 |
| Height, cm | 0.016 | 0.004 | <0.0001 |
| Waist Circumference, cm | 0.009 | 0.002 | <0.0001 |
| Cholesterol, mg/dL | 0.001 | 0.001 | 0.435 |
| High Density Lipoprotein, mg/dL | -0.000 | 0.002 | 0.991 |
| Low Density Lipoprotein, mg/dL | 0.001 | 0.001 | 0.338 |
| Triglycerides, mg/dL | -0.000 | 0.000 | 0.631 |
| Glucose, mg/dL | 0.009 | 0.003 | 0.001 |
| Insulin, mg/dL | 0.012 | 0.004 | 0.001 |
| Current Cigarette Smoker | -0.205 | 0.098 | 0.037 |
| Antihypertensive Medication Use | 0.132 | 0.057 | 0.020 |

Table 3.4 Multivariable linear regression of factors associated with common carotid artery adventitial diameter

| | Model | | |
|---|---|---|---|
| | β | Standard Error | p-value |
| Hormone Therapy Use | -0.137 | 0.047 | 0.004 |
| Age, years | 0.013 | 0.008 | 0.106 |
| African American Race | -0.049 | 0.074 | 0.503 |
| Pulse Pressure, mmHg | 0.008 | 0.002 | 0.0002 |
| Weight, kg | 0.012 | 0.002 | <0.0001 |
| Current Cigarette Smoker | -0.180 | 0.093 | 0.052 |

# 4.0 ASSOCIATIONS OF SLEEP QUALITY AND SLEEP APNEA RISK WITH SUBCLINICAL CARDIOVASCULAR DISEASE IN YOUNG ADULTS

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## 4.1 INTRODUCTION

Sleep apnea is a cycle of disordered breathing when the airway is narrowed or closed causing disrupted and fragmented sleep. It is associated with cardiovascular disease, obesity, high blood pressure and diabetes[1-9]. A small (n=24), all-male, randomized-control trial of severe obstructive sleep apnea patients showed evidence that 4-month treatment with the standard of care for sleep apnea (continuous positive airway pressure) reduced daytime and nighttime diastolic blood pressure, C-reactive protein and carotid-femoral pulse wave velocity[10]. This implies that standard treatment of sleep apnea in men with severe obstructive sleep apnea may improve vascular health. The majority of research on sleep apnea has been in children, adolescents, middle [4, 6, 11, 12] and older adults[13]; however, few studies have assessed young adults between 20 and 45[8].

Snoring is a form of sleep disordered breathing and may also be a marker of undiagnosed sleep apnea and its cardiovascular consequences. Snoring[14-17] and other types of sleep disordered breathing (often associated with sleep apnea)[1, 6-8, 18-20] have been linked to CVD.

There is a U-shaped relationship between higher risk of all-cause mortality and short sleep duration (<6 hours) and long sleep duration (>8 hours)[21]. Data from two cohort studies of men and women from the United States and the United Kingdom found that short sleep duration (<6 hours) was correlated to obesity and poor health, while long sleep duration (>8 hours) was associated with comorbidities[22]. An observational cohort study of young adults (35-47 years) with 5-year follow-up found that those with longer sleep duration had a reduced risk of coronary artery calcium[23].

The purpose of this study is to determine whether baseline sleep quality and sleep apnea risk were associated with measures of vascular health in young adults, 20-45 years old in an ongoing clinical trial. The measures used were carotid-femoral pulse wave velocity (cf-PWV), common carotid artery intima media thickness (IMT), adventitial diameter (AD) and lumen diameter (LD).

## 4.2 METHODS

### 4.2.1 Study Population

The Slow Adverse Vascular Effects (SAVE) study is a single-center, randomized-controlled, clinical trial designed to determine the vascular effects of a lifestyle intervention for weight loss, increased physical activity and sodium reduction in overweight and moderately obese young adults. The study recruited over 300 eligible men and women from Allegheny County, Pennsylvania and surrounding counties between March 2007 and May 2009 using direct mailing as the primary recruitment method. Eligible participants were between 20 and 45 years of age, overweight or obese (a body mass index (BMI) between 25.0 and 39.9 $kg/m^2$) and physically inactive. Adults were ineligible if they were exercising $\geq$ 3 hours per week for at least 8 of the past 12 months, diabetic, hypertensive, on medication to lower cholesterol, on vasoactive medications or vasoactive devices, pregnant or breast feeding or unable to comply with the intervention. The study was approved by the University of Pittsburgh's Institutional Review Board and a Data Safety Monitoring Board that met twice a year.

### 4.2.2 Screening

Each potential participant completed a telephone screen to determine eligibility based on age, estimated BMI (based on self-reported height and weight), medication use, and physical activity history. Those eligible based on the telephone screen were scheduled for a clinic screening visit.

At the screening visit participants gave informed consent for screening. Volunteer contact information, and a brief demographic form were returned and medication use was reported. The demographic form provided self-reported date of birth, gender, race, ethnicity, history of cigarette smoking, and history of alcohol use.

Weight was measured in kilograms with a balance scale. Height was measured in centimeters by a stadiometer and BMI was calculated as weight in kg/ height in $m^2$. Waist circumference was measured twice against the skin in centimeters at the narrowest circumference between the ribs and the iliac crest. If the waist circumference measures were within two centimeters the values were averaged for waist circumference; if they were not waist circumference was re-measured a third time and the two closest measurements were averaged. Blood pressure was measured after the participant sat quietly for 5-minutes with their feet flat on the floor using a standard protocol with a mercury

sphygmomanometer. Three measurements were taken 30 seconds apart; the average of the last two measurements was used.

### 4.2.3 Laboratory Tests

A 12-hour fasting blood draw was completed at the screening visit for each participant. Serum glucose was quantitatively determined by an enzymic determination procedure similar to that described by Bondar and Mead[24]. Total cholesterol was determined using the enzymatic method of Allain et al[25]. High density lipoprotein (HDL) cholesterol was determined after selective precipitation by heparin/manganese chloride and removal by centrifugation of very low density (VLDL) and low density lipoprotein (LDL)[26]. The HDL cholesterol was measured enzymatically. Triglycerides (TG) were determined enzymatically using the procedure of Bucolo et al[27]. LDL cholesterol was calculated indirectly using the Friedewald[28] equation: $LDL_c$ = Total cholesterol - $HDL_c$ - 0.2 (total TG) (Not used if total TG > 400 mg/dL).

### 4.2.4 Sleep Questionnaires

Sleep habits, duration and quality including snoring were assessed using two self-administered questionnaires: the Pittsburgh Sleep Quality Index (PSQI)[29] and Berlin Sleep Questionnaire (BSQ)[30].

The PSQI was developed to distinguish good and poor sleepers based on perceived sleep quality[29]. The self-administered questionnaire has 26-items, and is simple to interpret. The questionnaire has been shown to be reliable and valid[31, 32]. Each of the seven categories is scored and a composite score is determined with a range from 0 to 21. Validation of the questionnaire identified a low score ($\leq$ 5) is associated with good sleep quality and a high score (> 5) is associated with poor sleep quality. This analysis defines good vs. poor sleep quality using the $\leq$ 5 cut point.

The BSQ was designed to determine sleep apnea risk[29]. It is self-administered and simple to administer and interpret. The questionnaire has been shown to be reliable and valid[33]. The questionnaire has three categories: habitual snoring, tiredness during the day or daytime dysfunction, and obesity and hypertension. The BSQ defines obesity as BMI $\geq$ 30 kg/m$^2$. The categories are scored and summed for a total score that ranges from 0 to 3[29]. A score $\leq$ 1 reflects a low risk for sleep apnea and a score > 1 reflects a high risk for sleep apnea. The data is evaluated based on these two cut points: low and high risk.

### 4.2.5 B-mode Ultrasound Measurements

B-mode ultrasound measurements were scheduled as part of baseline visits. All ultrasound tests were conducted by trained, certified lab technicians at the University of Pittsburgh Department of Epidemiology's Ultrasound Research Laboratory (URL). All URL sonographers were re-certified every six months.

Cf-PWV was measured using the Complior Pulse Wave Velocity Machine (Artect, VAB00001). Cf-PWV was measured by taking simultaneous recordings of the pulse waves from the right common carotid and right femoral artery using mechanotransducers while the participant was in supine position. Cf-PWV was calculated by dividing the distance between transducers by the time of pulse wave travel. Output from the transducers was captured and processed using the Complior SP system. Three data collection runs were performed, each obtaining an average of ten pairs of simultaneously recorded pulse waves. The between-tech intraclass correlation coefficient was 0.78-0.91 and within-tech intraclass correlation coefficient were 0.88-0.95 for cf-PWV.

Common carotid artery IMT, AD and LD were measured using the Antares machine (Siemens, Sonoline) with a 5-MHz linear array transducer. At the baseline visit, images were digitalized from the near and far walls of the distal common carotid artery (one centimeter proximal to the carotid bulb), the

carotid bulb (the point at which the near and far walls of the common carotid artery were no longer parallel to the tip of the flow divider), and the first 1 cm of the internal carotid artery (from the tip of the flow divider). IMT was defined as the distance from the lumen-intimal interface to the medial-adventitial interface. AD was defined as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interfaces on the far wall. LD was defined as the distance from the intima-lumen interface of the near wall and the lumen-intima interface of the far wall. The interfaces were traced electronically over the respective areas and a computer generated measurement was obtained for each pixel in the area of interest; these measurements were averaged to determine the IMT, AD and LD used for this analysis. The between-tech intraclass correlation coefficient was 0.89-0.99 for average IMT.

### 4.2.6 Statistical Methods

All analyses were based on cross-sectional baseline data using SAS v9.1 (SAS Institute, Cary, NC). Preliminary analysis showed the relationship between sleep measures and CVD measures differed by sex (data not shown), therefore all analyses were stratified by gender. The mean (for normal continuous variables), the median (for non-parametric continuous variables), or the percent (for categorical variables) were determined for the demographic, anthropometric

and health characteristics of the population. The Chi-square test for categorical and the Wilcoxon rank sum test for continuous variables were used to test for differences in sleep characteristics and subclinical CVD measures between men and women. General Linear Modeling was used to test whether self-reported sleep quality or sleep apnea risk were related to the subclinical CVD measures controlling for potential confounders age, and systolic blood pressure. It was decided a priori if sleep quality, determined by the PSQI, or sleep apnea risk, determined by the BSQ, were related to any subclinical CVD measure, additional analyses would be performed to determine which aspects of sleep quality or risk for sleep apnea were related to the subclinical CVD measures.

## 4.3 RESULTS

Most of the participants were female (77.4%) and Caucasian (84.0%). The average age was 38 years and mean BMI was 33.1 kg/m$^2$ (Table 4.1). The percent of those with poor sleep quality was not statistically different in men and women (Table 4.2). However, men reported a shorter sleep duration than women (p=0.0055, Table 4.2). A higher percent of men had high risk sleep apnea scores (63%) than women (49%) (p=0.0281, Table 4.2). As expected men had higher median cf-PWV (7.9 mm/s, 7.6 mm/s), thicker IMT (0.62 mm, 0.59 mm), larger AD (7.4 mm, 6.8 mm) and larger median LD (6.1 mm, 5.6mm) than women (all p<0.05, Table 4.2).

There was no difference in subclinical CVD measures between those who had poor sleep quality and those who had good sleep quality determined by the PSQI (data not shown).

Women at high risk for sleep apnea had higher IMT, larger AD and higher cf-PWV than women at low risk for sleep apnea (data not shown). These results were attenuated and lost significance when the model adjusted for age and SBP (Table 4.3). In men, AD and LD were significantly larger in those at high risk

for sleep apnea; these results remained robust after adjustment for age and SBP (Table 4.3).

Next, snoring (at least once/month vs. not snoring in the last month) and the "tiredness during the day or daytime dysfunction" component of the BSQ, were examined to determine their relationship with subclinical CVD measures.

Those who reported snoring had significantly worse subclinical CVD measures than those who did not report snoring; the tiredness during the day/daytime dysfunction component was not related to the subclinical CVD measures (data not shown). Snorers were significantly older and had higher BMI and blood pressure than non-snorers (all $p < 0.05$, data not shown).

Women had no differences in IMT, AD, LD or cf-PWV based on snoring status with or without adjustment for age and SBP (Table 4.4). In men, AD and LD were higher in the snorers than the non-snorers after adjustment for age and SBP (Table 4.4).

## 4.4 DISCUSSION

This study of overweight and obese young adults found that even after adjustment for age and SBP, men at high risk for sleep apnea and men that snored had significantly larger AD and LD than men at low risk for sleep apnea and non-snorers. In women, none of the subclinical CVD measures were significantly different by sleep apnea risk or snoring status after adjustment for age and SBP. Baseline self-reported sleep quality was not related to any of the subclinical CVD measures assessed in the SAVE participants.

The analyses investigating aspects of the BSQ and subclinical CVD measures showed snoring was significantly related to a worse cardiovascular profile and poorer vascular health. Snorers had significantly higher BMI and higher blood pressure. Obesity and high blood pressure are associated with sleep apnea. Lee et al objectively measured snoring in a cross-sectional study of Australian adults (mean age 58.2±7.9); objectively measured heavy snoring was associated with higher BMI and greater prevalence of hypertension[34]. Heavy snorers also had a higher prevalence of carotid plaque measured by B-mode ultrasound[34]. In the current study, AD and LD were significantly larger in male snorers; but not in females. There were few women in the moderate (n=10) and heavy (n=5) snoring strata compared to men (n=15 in both groups), thus Lee et

al did not evaluate the results by gender[34]. The differential effect of snoring on the vasculature by gender in the current study suggests further research is needed.

Snoring may be a risk factor for carotid disease. Animal studies in rats and rabbits help explain some biological mechanisms between snoring and carotid disease. These studies provide evidence that vibrational pressure waves to travel from the upper airway to the carotid artery[35], and chronic vibrational energy damages vascular cells[35-38]. The vibrations act as mechanical stress to the artery. Managing arterial stress is the role of the adventitia which may explain the significance of AD observed between snorers and non-snorers in men in the current study. These vibrations also destroy vascular smooth muscle cells[38], the main component of the media layer of the artery wall[39] and damage endothelial cells[40] that compose the intima or inner layer of the artery. Thus, the independent association of AD with snoring after controlling for age and SBP in the current study is supported by the biological mechanisms observed in animals.

Snoring is a form of sleep disordered breathing and may also be a marker of undiagnosed sleep apnea and its cardiovascular consequences. Snoring[14-17] and other types of sleep disordered breathing (often associated with sleep apnea)[1,6-8, 18-20] have been linked to CVD. A subsample of the Nurses' Health

study found that occasional snorers had a 20% higher risk of CVD, and regular snorers a 33% increased risk of CVD than non-snorers in an 8 year longitudinal study of women who were free of CVD at baseline[14].

Strengths of this study were that the population was a well defined sample of community-dwelling young adults; assessment of subclinical measures was of high quality by certified, trained sonographers & technicians. Limitations of this study were the subjective nature of the questionnaires and the small number of men, and simple snoring versus sleep apnea cannot be distinguished from the BSQ.

In conclusion, these findings suggest overweight and obese young adults at high risk for sleep apnea and those who snore have early adaptive changes to the vasculature linked to adverse vascular health.

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## 4.7 DISCLOSURES

There are no disclosures.

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## 4.9 TABLES FOR CHAPTER 4

Table 4.1 Baseline demographic, anthropometric and health characteristics

| | **Women (n = 270)** | **Men (n = 79)** |
|---|---|---|
| | mean ± standard deviation, median ($25^{th}$, $75^{th}$ percentile) or percent | |
| Age, years | 38.0 ± 6.1 | 37.3 ± 6.1 |
| Caucasian, % | 81.1 | 93.7 |
| Body Mass Index, $kg/m^2$ | 32.7 ± 3.8 | 33.5 ± 3.7 |
| Systolic Blood Pressure, mmHg | 112.4 ± 10.6 | 117.1 ± 9.1 |
| Diastolic Blood Pressure, mmHg | 71.9 ± 8.5 | 76.6 ± 8.3 |
| Glucose, mg/dL | 94.2 ± 8.2 | 99.6 ± 7.3 |
| Current Cigarette Smoker | 8.9 | 11.4 |
| Total Cholesterol, mg/dL | 201.0 (178,226) | 200.0 (175,222) |
| HDL Cholesterol, mg/dL | 53.7 (45,62) | 42.5 (37,47) |
| LDL Cholesterol, mg/dL | 121.5 (98,144) | 124.0 (105,149) |
| Triglycerides, mg/dL | 105.5 (76,156) | 152.0 (110,209) |

HDL High Density Lipoprotein, LDL Low Density Lipoprotein

Table 4.2 Sleep and subclinical CVD characteristics by gender

| | **Women (n = 270)** | **Men (n = 79)** | |
|---|---|---|---|
| | median (25th, 75th percentile) or % | | p-value |
| Amount of sleep*, hours | 7.0 (6.0,7.5) | 6.5 (6.0,7.0) | 0.006 |
| PSQI Poor Quality Sleep†, % | 48.5 | 53.2 | 0.468 |
| High Risk BSQ score†, % | 49.3 | 63.3 | 0.028 |
| Snorers†, % | 46.0 | 67.1 | 0.001 |
| Intima media thickness*, mm | 0.58 (0.54,0.63) | 0.62 (0.57,0.67) | 0.001 |
| Adventitial Diameter*, mm | 6.8 (6.5,7.1) | 7.4 (7.1,7.6) | <0.0001 |
| Lumen Diameter, mm | 5.6 (5.3,5.9) | 6.1 (5.9,6.4) | <0.0001 |
| Cf-Pulse Wave Velocity*, mm/s | 7.6 (6.9,8.4) | 7.9 (7.2,8.6) | 0.017 |

* Wilcoxon rank sum test, †Chi-square test,

BSQ Berlin Sleep Questionnaire, cf carotid-femoral, CVD Cardiovascular Disease,

PSQI Pittsburgh Sleep Quality Index

Table 4.3 Low risk and high risk for sleep apnea & subclinical CVD measures by gender

| | **Women** | | | **Men** | | |
|---|---|---|---|---|---|---|
| | **Low Risk SA (n=137)** | **High Risk SA (n=133)** | | **Low Risk SA (n=29)** | **High Risk SA (n=50)** | |
| | median (25th, 75th percentile) | | p-value* | median (25th, 75th percentile) | | p-value* |
| IMT, mm | 0.56 (0.53,0.62) | 0.60 (0.44,0.66) | 0.434 | 0.61 (0.54,0.66) | 0.63 (0.57,0.67) | 0.165 |
| AD, mm | 6.7 (6.4,7.0) | 6.8 (6.5,7.1) | 0.144 | 7.4 (6.9,7.5) | 7.4 (7.2,7.7) | 0.012 |
| LD, mm | 5.6 (5.3,5.8) | 5.6 (5.3,5.9) | 0.177 | 6.1 (5.7,6.3) | 6.1 (6.0,6.4) | 0.020 |
| cf-PWV, cm/s | 7.3 (6.8,8.2) | 7.8 (7.2,8.7) | 0.178 | 7.5 (7.2,8.2) | 8.1 (7.5,8.7) | 0.276 |

*p-value from a general linear model controlling for age and systolic blood pressure

AD Adventitial Diameter, cf-PWV carotid-femoral Pulse Wave Velocity, CVD Cardiovascular Disease, IMT Intima Media Thickness, LD Lumen Diameter, SA Sleep Apnea

Table 4.4 Snoring status & subclinical CVD measures by gender

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | **Non-snorers (n=146)** | **Snorers (n=124)** | | **Non-snorers (n=26)** | **Snorers (n=53)** | |
| | median (25th, 75th percentile) | | p-value* | median (25th, 75th percentile) | | p-value* |
| IMT, mm | 0.57 (0.53, 0.62) | 0.60 (0.54, 0.65) | 0.738 | 0.61 (0.54, 0.67) | 0.62 (0.57, 0.67) | 0.684 |
| AD, mm | 6.7 (6.4, 7.0) | 6.8 (6.5, 7.1) | 0.628 | 7.4 (6.9, 7.5) | 7.4(7.2, 7.7) | 0.034 |
| LD, mm | 5.6 (5.3, 5.9) | 5.6 (5.3, 5.9) | 0.672 | 6.1 (5.7, 6.3) | 6.1 (5.9, 6.4) | 0.030 |
| cf-PWV, cm/s | 7.5 (6.8, 8.3) | 7.7 (7.1, 8.6) | 0.909 | 7.7(7.3, 8.4) | 8.0 (7.2, 8.6) | 0.697 |

* p-value from a general linear model controlling for age and systolic blood pressure

AD Adventitial Diameter, cf-PWV carotid-femoral Pulse Wave Velocity, CVD Cardiovascular Disease, IMT Intima Media Thickness, LD Lumen Diameter

# 5.0 SUBCLINICAL ATHEROSCLEROSIS AND PHYSICAL ACTIVITY IN OLDER ADULTS: LIFE RANDOMIZED PILOT STUDY

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## 5.1 INTRODUCTION

Atherosclerosis is a disease of the arteries that begins as a fatty cholesterol streak in the inner wall of the artery; regression or further development is dependent upon several factors. Subclinical atherosclerosis is asymptomatic and precedes clinical cardiovascular disease (CVD) events such as myocardial infarction[1-3] and stroke[2, 4-7]. Subclinical atherosclerosis can be detected using several non-invasive, reliable and valid, B-mode ultrasound measures including pulse wave velocity (PWV)[8, 9], intima media thickness(IMT)[10, 11], adventitial diameter(AD)[12, 13] and lumen diameter(LD)[12, 14].

Although, thickening of the intima-media layers[15] and arterial stiffening[16, 17] are characteristic of normal aging; they are also associated with atherosclerosis[10], CVD risk factors and CVD[7]. There is evidence that behavioral factors such as smoking[18, 19], and poor dietary habits[19] influence atherosclerotic progression. Physical activity (PA) has been associated with CVD benefit[20-22]. Potential mechanisms are the influence it has on arterial remodeling. Cross-sectional studies of exercise and physical activity show higher PWV in sedentary or less active groups, indicating more arterial stiffness than active groups[17, 23, 24]. Longitudinal studies have found no difference in change in PWV between active and less active groups[25, 26]. Inconsistent results from prior studies may be

explained by differing populations and control groups, type, intensity and duration of the PA intervention.

The Lifestyle Interventions and Independence for Elders (LIFE) project was a randomized controlled pilot study designed to determine if a PA intervention compared to a successful aging (SA) education-only control group could improve physical function and decrease disability in sedentary older adults at risk for mobility disability[27]. Post intervention participants in the PA group had significantly better performance on measures of physical function, lower rates of mobility disability, and were more physically active than those in the SA group[28]. An ancillary study of LIFE was developed to evaluate whether the PA intervention would affect subclinical CVD progression. The main outcome was change in arterial stiffness measured by carotid-femoral PWV. Change in common carotid artery (CCA) IMT, AD and LD were secondary outcomes.

## 5.2 METHODS

### 5.2.1 Population

Four hundred twenty-four eligible sedentary men and women were recruited between April 2004 and February 2005 at four field centers located at the Cooper Institute in Dallas, TX, Stanford University in Palo Alto, CA, University of Pittsburgh in Pittsburgh, PA and Wake Forest University in Winston-Salem, NC for the LIFE randomized controlled pilot study[28]. Eligible participants were sedentary (reported fewer than 20 minutes/week of structured physical activity in the last month), 70-89 years of age, able to walk 400-meters, at risk for mobility disability (had a Short Physical Performance Battery (SPPB) score $\leq$9), living in one of the study areas, willing to provide informed consent, and able to complete a behavioral run-in[28]. The behavioral run-in was a 1-week, pre-randomization test of compliance; the participants were asked to complete a daily food diary of the quantity and kind of fruits and vegetables consumed and a physical activity diary of the types and frequency of physical activity[27]. Successful completion of the behavioral run-in was defined as completing the diary at least 6 of the 7 days[27]. Participants were randomized to the moderate PA group or the SA education-only control group after successfully completing the run-in and

baseline assessment. The study was approved by the institutional review boards of each institution.

In order to study how a PA intervention influenced the risk of CVD all LIFE participants at the University of Pittsburgh were asked to participate in an ancillary study which included measures of subclinical CVD. Of 106 participants, 52 agreed to participate and provided informed consent.

### 5.2.2 Screening, Baseline Assessment

The screening process consisted of a telephone interview, SPPB, a medical screening, a 400-meter walk test and the behavioral run-in[29]. First, the telephone screening obtained information for initial eligibility: verbal informed consent to be screened, age, zip code, physical activity level and health status[29]. Eligible volunteers were then scheduled for a clinic visit where written informed consent was obtained and the SPPB was completed[29]. Volunteers with SPPB score of >10 were asked to undergo medical screening[29]. Written informed consent for the main study, demographic information, socioeconomic status, lifestyle habits, physical function, physician-diagnosed disease information, medication inventory and health-related quality of life were self reported and anthropometric measurements, blood pressure, radial pulse, grip strength, an electrocardiogram, physical activity (assessed by the Community Healthy

Activities Model Program for Seniors (CHAMPS)[30] questionnaire) and cognition (assessed using the Mini-Mental State Examination) were objectively measured at the medical screening visit[27, 28]. Abnormal electrocardiogram tests were reviewed by the field center physician[29] who determined whether the volunteer was eligible. Volunteers deemed eligible based on the medical screening were then given the 400-meter walk test[29]. Next, eligible volunteers who successfully completed the 400-meter walk (<15 minutes, without the use of cane or assistive device) were given instructions for completing the compliance run-in; those with unsuccessful completion were given one additional opportunity. Randomization occurred after successful completion of the run-in and was stratified by gender and site.

### 5.2.3 6-month Follow-up Assessment

The six-month assessment consisted of the same measures completed at the baseline visit minus collection of demographic information, socioeconomic status, and lifestyle habits, the Mini-Mental State Examination, physical exam, and electrocardiogram[27]. The subclinical CVD measures were scheduled the same day as the 6-month follow-up clinic assessment for the main LIFE pilot study.

### 5.2.4 Ultrasound Measures

Carotid-femoral PWV was measured by using two 8-10 MHz unidirectional transcutaneous Doppler flow probes model 810-a (Parks Medical Electronical, Aloha, OR) placed at the right carotid and femoral arteries with the participant in the supine position[9]. Simultaneous recordings of the pulse waves and electrocardiogram output from the carotid and femoral arteries were obtained and stored on a computer for later reading[9]. PWV was calculated by dividing the distance between transducers by the time of pulse wave travel. When the recordings were read, three cycles of pulse waves were averaged to obtain PWV.

CCA IMT, AD, and LD were assessed by B-mode ultrasound using a Sonoline Antares duplex scanner (Siemens, Malvern, PA) with a 5-MHz linear array transducer. Images were taken on the right CCA of the near and far walls of the distal CCA 1 cm proximal to the carotid bulb. IMT was defined as the distance from the lumen-intimal interface to the medial-adventitial interface. Computer generated measurements were obtained from the electronically traced lumen-intimal and medial-adventitial interfaces over the areas of interest; these measurements were averaged to determine IMT. AD was defined as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interfaces on the far wall. Computer generated measurements were

obtained from the electronically traced adventitial-medial and medial-adventitial interfaces; these measurements were averaged for the determination of AD. LD was defined as the distance from the intima-lumen interface of the near wall to the lumen-intima interface of the far wall. Computer generated measurements were obtained from the electronically traced intima-lumen and lumen-intima interfaces; these measurements were averaged to determine LD.

### 5.2.5 Intervention

The LIFE-pilot interventions are described in more detail elsewhere[27]. The PA intervention had three phases: adoption, transition and maintenance. A one-on-one, 45-minute introduction session was conducted by an exercise physiologist to explain the intervention and answer questions prior to the adoption phase[27]. The PA group participated in one-on-one, center based, 60 minute sessions of aerobic (40 minutes), strength (10 minutes), balance and flexibility (10 minutes) exercises three times a week for the 1st eight weeks of the intervention[27]. At week four home based exercises were incorporated once a week through week five, and twice a week in weeks six through eight. During the transition period, weeks 9-28, participants attended two more center based sessions and completed three home based sessions per week[27]. There were also 10 group counseling sessions between weeks one and eight to emphasize physical

activity and the prevention of mobility disability[27]. The maintenance phase included monthly center based sessions and home sessions 5 times a week until the end of the 12-month intervention[27]. Participants were encouraged to participate in at least 150 minutes of moderate intensity physical activity per week[27]. Walking was the main mode of exercise for its ease and safety.

The SA group met weekly for the first 26 weeks then monthly until the 12-month follow-up. The intervention was given in a small group setting; participants were provided relevant health information for older adults including nutrition, medication use, foot care and preventive care services[27, 28]. In addition, upper extremity stretching exercises were led by the group leader for 5-10 minutes at the end of each session[27, 28].

### 5.2.6 Statistical Methods

Wilcoxon-rank sum tests and Chi-square tests were used to determine differences in baseline characteristics between the intervention groups. Wilcoxon signed rank tests were used to evaluate progression of subclinical CVD within groups. Change in subclinical measures was determined by subtracting baseline values from 6-month values. General Linear Models (GLM) were used to assess between group differences in change in subclinical measures. Models were run for each subclinical measure adjusting for the applicable baseline

subclinical measure, race, gender, age, BMI, pulse pressure, history of hypertension and history of diabetes. A p-value < 0.05 was considered statistically significant. All analyses used intention-to-treat group assignments and were completed using SAS v9.1 (SAS Institute Inc., Cary, NC).

Post-hoc sample size and power calculations were made using PASS 2008 (NCSS, Kaysville, UT) to determine the effect size that could be detected given the sample size of the PA and SA groups. The final sample size for this analysis was 38.

## 5.3 RESULTS

Fourteen of the 52 participants were excluded from analysis due to unusable baseline or follow-up subclinical measures. The reasons for incomplete data were difficulty obtaining usable images due to an inability of the participant to lie flat (n=11), mechanical error (n=2) and refusal to complete follow-up measures (n=1).

The majority of the sample was female (73.4%) and hypertensive (55.3%); 15.8% were African American, 18.4% had diabetes and none were current smokers. The median (range) age was 78 (70-85) years and BMI was 29.2 kg/m$^2$ (20.1-44.1). Eighteen participants were in the PA intervention group and 20 participants were in the SA education-only group. Due to the small sample size there was inadequate power to detect clinically relevant differences between groups in demographic, anthropometric, CVD risk factors or subclinical measures at baseline (Table 5.1). (E.g. a sample size of 18 and 20 allowed us to detect an effect size of 0.91 or a difference in proportions of approximately 40-45% with 80% power.) As a result, none of the variables were statistically different between groups (data not shown); however with a larger sample size, differences in age, gender, race, pulse pressure, BMI, history of hypertension and history of diabetes may have been seen. Due to our inability to determine

statistically significant relationships between these variables and the groups, we adjusted all analyses for these covariates.

PWV appeared to decrease between baseline and 6-month follow-up in the PA group, although the difference did not reach statistical significance (Table 5.2; p=0.08). Although median change in the SA control group was also lower, about half the group had higher values at 6 months. As a result, this change was not statically significant (Table 5.2; p=0.64). The difference in change in PWV between groups was significant after adjustment for baseline PWV, age, gender, race, pulse pressure, BMI, history of hypertension and history of diabetes (Figure 5.1).

The PA group had increased AD (p=0.03) and LD (p=0.02) between baseline and 6-month follow-up while the SA group had decreased AD (p=0.03) and LD did not differ significantly (Table 5.2). The two groups had significantly different changes in AD (p=0.003) and LD (p=0.02) in fully adjusted models (Figures 5.2 and 5.3).

No change was observed in either group between baseline and six month follow-up IMT (Table 5.2). Change in IMT between groups was also insignificant (Figure 5.4).

## 5.4 DISCUSSION

In this study of older adults progression of arterial stiffening (measured by PWV) during the 6-month intervention differed by intervention group. Specifically, the PA group had significantly lower PWV than the SA control group. The PA group's regression of arterial stiffening (median decrease of 257 cm/s) was clinically relevant. Although this study found no change in PWV in the SA group (median decrease 28 cm/s), longitudinal studies of PWV progression have reported increases of 11.1 to 68.3 cm/s per year in normotensive and treated hypertensive participants[31] and those with impaired glucose, high blood pressure, both or neither[32]. The PWV findings from the current study are in line with the several cross-sectional studies which have found endurance trained adults and active healthy adults have lower PWV than their sedentary counterparts[17, 23, 24]. Endurance trained older men from the Baltimore Longitudinal Study of Aging[17] and endurance trained postmenopausal women[24] had 30% lower PWV than their sedentary counterparts in two cross-sectional studies[17, 24]. A cross-sectional study of healthy men and women who were either sedentary or active (practiced yoga at least twice a week or aerobic exercise at least three times a week over the past year) found that the active participants had significantly lower PWV than the sedentary participants[23]. However, two intervention studies have found null results. A randomized cross-

over study with 8 weeks of moderate intensity cycling and 8 weeks of sedentary behavior was conducted in ten adults with untreated isolated systolic hypertension (systolic blood pressure >150 mmHg and diastolic blood pressure <90 mmHg) did not observe differences in PWV after the sedentary phase compared to the PA phase[25]. Similarly, a randomized study comparing a walking intervention and an active control group (sodium restriction) in postmenopausal women with untreated elevated blood pressure (systolic blood pressure 130-159 mmHg, diastolic blood pressure $\geq$ 99 mmHg) did not observe a reduction in PWV in the walking group while those in the active control (sodium restriction) did[26]. However, there were important differences in these studies and the current study. These studies were shorter in duration[25],[26]. This may suggest changes in stiffness require sustained physical activity for more than three months. Both studies also studied untreated hypertensive individuals who may be more resistant to arterial changes from short-term increased physical activity than normontensive peers[25]. Additionally, the postmenopausal women in the sodium restriction control group had a large reduction in arterial stiffness[26]. Consequently, the effect of physical activity was not compared to sedentary behavior alone.

No prior studies, to our knowledge, have included AD in the assessment of PA and subclinical CVD. Thus, our expectations were the PA group would have better vascular health, reflected in smaller AD than the SA group. These

expectations were based on studies that showed smaller AD was associated with better vascular health in younger cohorts. Contrary to our expectations AD increased in the PA group and decreased in the SA group. This suggests the intervention may have affected vascular remodeling in ways not indicated by the more commonly used IMT. The adventitia is more sensitive to hemodynamic changes in the arterial environment than the media and intima layers of the artery[33].

LD also increased between baseline and six month follow-up in the PA group. LD changes observed in the PA group could reflect increased sheer stress, the tangential, mechanical force exerted on the vessel from the flow of blood on the lumen[34], due to increased blood flow induced by physical activity and exercise. A positive association between blood flow and femoral LD has been observed in a cross-sectional study comparing sedentary and endurance trained men ($r=0.39$, $p<0.001$) and a 3-month moderate endurance training intervention study of previously sedentary men ($r=0.62$, $p<0.0001$)[35].

There was neither within group change in IMT nor between group differences in change in IMT. Consistent with our results, two cross-sectional studies comparing athletes/physically active adults to sedentary adults found no relationship[36, 37]. Active adults who ate a healthy diet had lower IMT than sedentary adults who ate an unhealthy diet in a cross-sectional study[19]. This relationship was only

present in non smokers; diet and activity were not examined separately in this study[19].

The mechanisms explaining how physical activity and exercise are beneficial to arterial aging are not well established[38]. A proposed mechanism is that exercise and physical activity lower or reverse the effects of age-related vascular structural changes, but this mechanism has not been supported with data[38]. The beneficial effects of physical activity may be related to the improvement of cardiovascular risk factors such as improved lipid profiles and weight management[39]. A large prospective study of women suggested inflammatory markers like C-reactive protein and fibrinogen, and reduced blood pressure explain 33% and 27% of CVD risk reduction associated with physical activity[40].

The findings of this study must be interpreted with caution due to the small sample size and large variance in 6-month change yielding insufficient power for most analyses. Although activity was significantly higher in the PA group than the SA group[28], the large variation in 6-month change in arterial stiffening and diameter observed in both groups may be associated with the amount or intensity of physical activity or health behavior changes adopted by the groups. A wider variation of subclinical disease is observed with increasing age[9, 41-43]. The 70-89 year olds in this study have a much wider variance of values than studies of middle-aged adults. The goal of the ancillary study was to obtain complete

subclinical atherosclerosis measures on 52 participants; we were successful obtaining information on 73% of this sample for the measures of AD, LD and IMT but only 44% for PWV. The inability of some participants to lie flat was the main reason usable images could not be obtained; the problem was resolved with the purchase of a reclining chair. An additional limitation of the study is antihypertensive medication use was not assessed. Some antihypertensive medications are known to alter arterial stiffness[44, 45]. Thus, due to the high history of hypertension (50% in PA and 60% SA) it is possible there were group differences in antihypertensive medication use.

Some strengths of this study include: valid and reliable methods; the lab that performed the ultrasound measures consistently exhibits excellent reproducibility and procedures for continuous quality control [9, 45, 46]; the study population was well defined; and a novel measure of vascular health (AD) was used. No previous studies have evaluated the relationship between PA and AD which may provide information about vascular health and adaptation that is different than that indicated by IMT or LD alone.

In conclusion, despite a small sample size we found significant differences in change in PWV, AD and LD in older adults assigned to a PA intervention or SA control group. We observed that the PA group experienced regression of arterial stiffening but increased arterial diameters. A larger sample size is needed

to fully understand what these vascular changes mean in this population, to allow adjustment for confounders and to assess gender and racial differences. The phase 3, randomized, controlled LIFE study has been funded but currently does not include measures of subclinical atherosclerosis. Therefore, there may be an opportunity to evaluate this question in a larger sample of participants through an ancillary study where measures of subclinical atherosclerosis are obtained.

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## 5.7 DISCLOSURES

There are no disclosures.

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## 5.9 TABLES AND FIGURES FOR CHAPTER 5

Table 5.1 Baseline characteristics of randomized groups

| | **Physical Activity (n=18)** | **Successful Aging (n=20)** |
|---|---|---|
| | median ($25^{th}$, $75^{th}$ percentile) or % | |
| Age, years | 75.5 (72,80) | 78 (74,82) |
| Female, % | 66.7 | 80.0 |
| African American Race, % | 11.1 | 20.0 |
| Blood Pressure, mmHg | | |
| Systolic blood pressure | 139 (121,146) | 136 (123,148) |
| Diastolic blood pressure | 67 (59,76) | 68 (64,77) |
| Pulse pressure | 69 (61,81) | 64 (46,72) |
| Body mass index, $kg/m^2$ | 30.3 (28,35) | 28.9 (26,32) |
| Current Cigarette Smoker, % | 0 | 0 |
| History hypertension, % | 50.0 | 60.0 |
| History Diabetes, % | 27.8 | 10.0 |

Table 5.2 Baseline, 6-month and change in subclinical measures by intervention group

| | **Physical Activity** | | | | **Successful Aging** | | | |
|---|---|---|---|---|---|---|---|---|
| | Median (25th, 75th percentile) | | | | Median (25th, 75th percentile) | | | |
| | Baseline | 6-month | p-val* | Change | Baseline | 6-month | p-val* | Change |
| PWV | 1158 (965,1304) | 951 (820,1289) | 0.08 | -257 (-371,-6.55) | 1072 (896,1384) | 1063 (820,1328) | 0.64 | -28 (-359,181) |
| AD | 7.93 (7.37,8.86) | 8.02 (7.64,9.00) | 0.03 | 0.09 (-0.02,0.26) | 7.87 (7.39,8.15) | 7.78 (7.20,8.15) | 0.03 | -0.16 (-0.28,0.02) |
| LD | 5.95 (5.65,6.57) | 6.03 (5.87,6.71) | 0.02 | 0.19 (-0.07,0.27) | 5.78 (5.49,6.07) | 5.77 (5.39,6.15) | 0.20 | -0.07 (-0.27,0.09) |
| IMT | 0.99 (0.86,1.13) | 0.95 (0.85,1.05) | 0.25 | -0.02 (-0.05,0.01) | 1.00 (0.92,1.08) | 0.97 (0.90,1.11) | 0.47 | -0.01 (-0.05,0.02) |

* Wilcoxon signed rank test comparing baseline and 6-month values

p-val p-value, PWV Pulse Wave Velocity, AD Adventitial Diameter, LD Lumen Diameter, IMT Intima Media Thickness

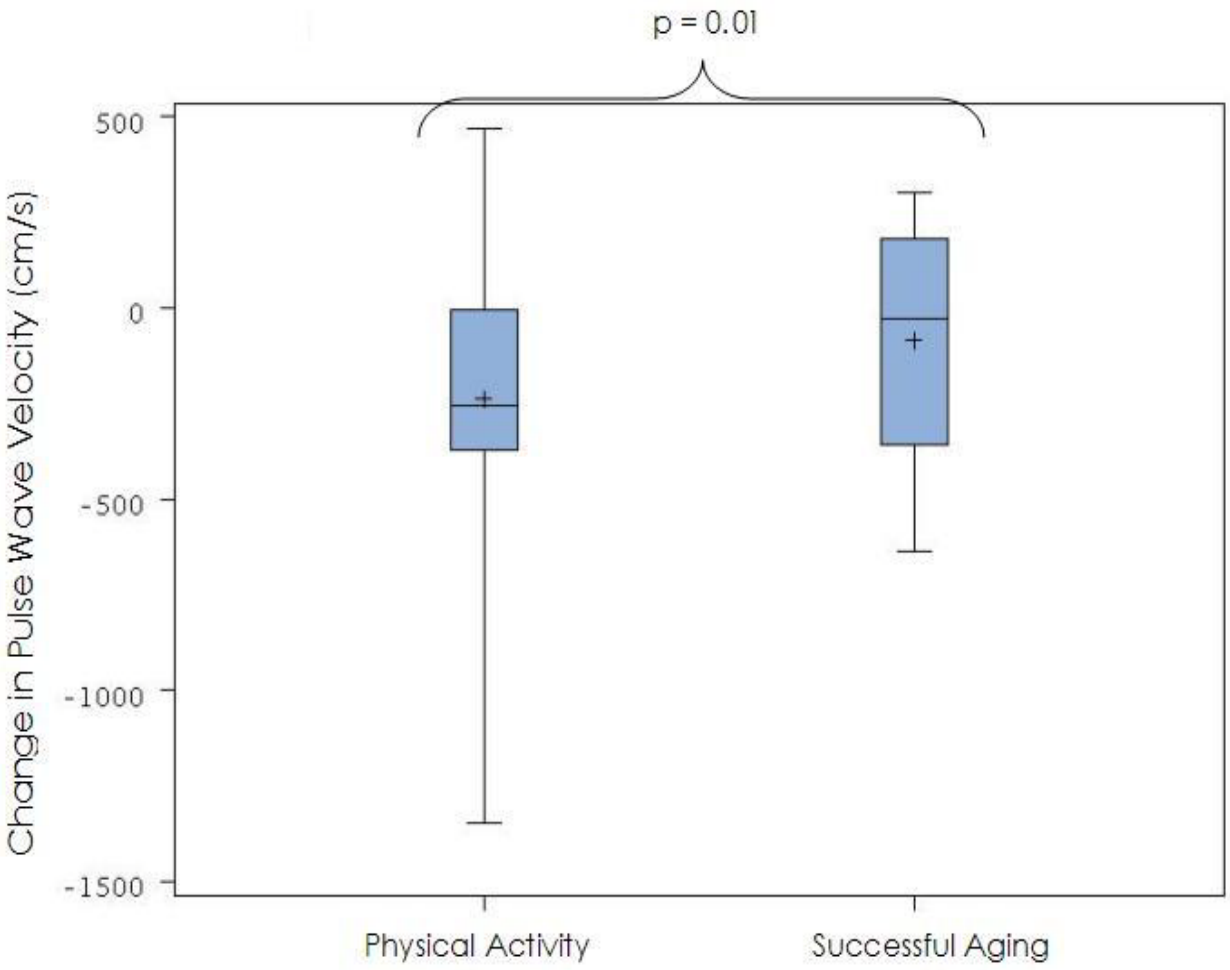


Figure 5.1 Box plot of change in pulse wave velocity by intervention group

P-value is from a general linear model controlling for baseline pulse wave velocity, race, gender, age, BMI, pulse pressure, history of hypertension and history of diabetes

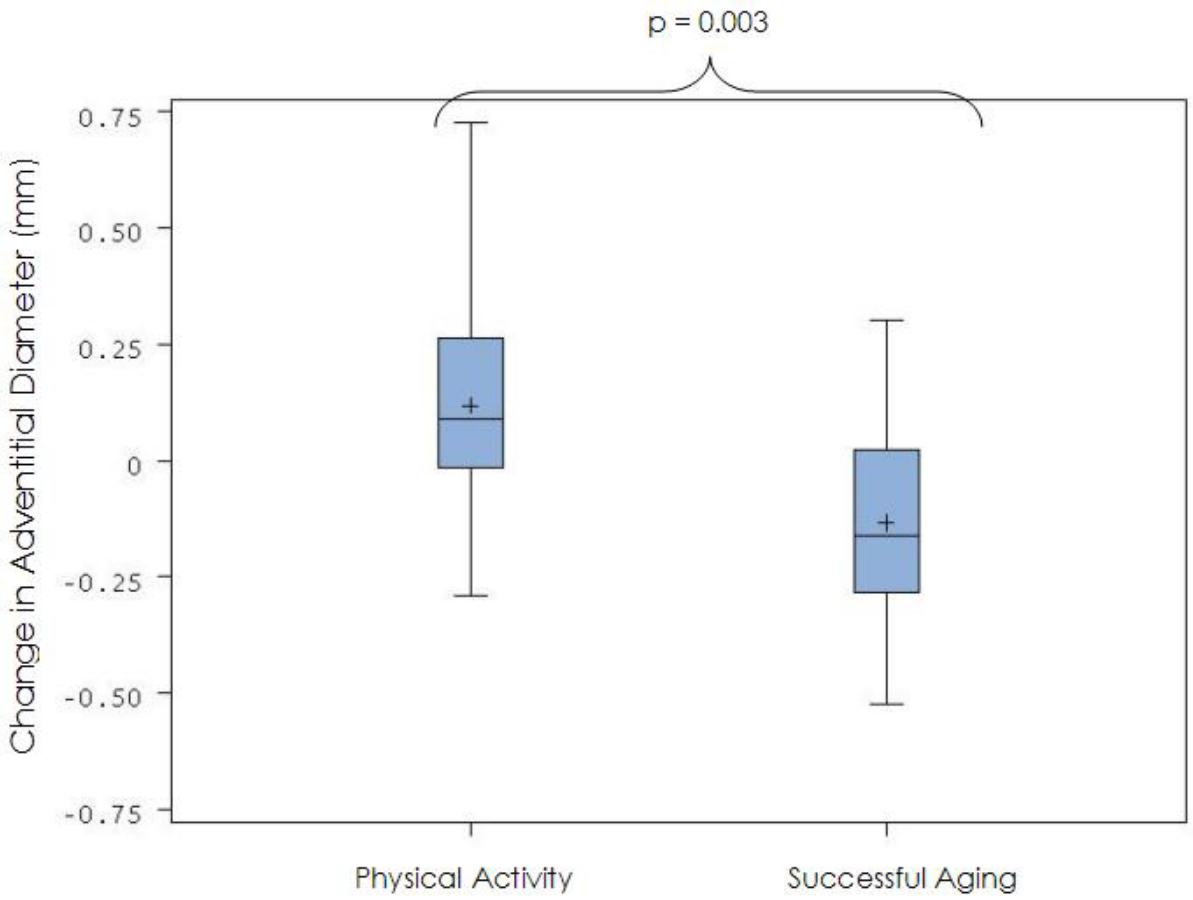


Figure 5.2 Box plot of change in adventitial diameter by intervention group

P-value is from a general linear model controlling for baseline adventitial diameter, race, gender, age, BMI, pulse pressure, history of hypertension and history of diabetes

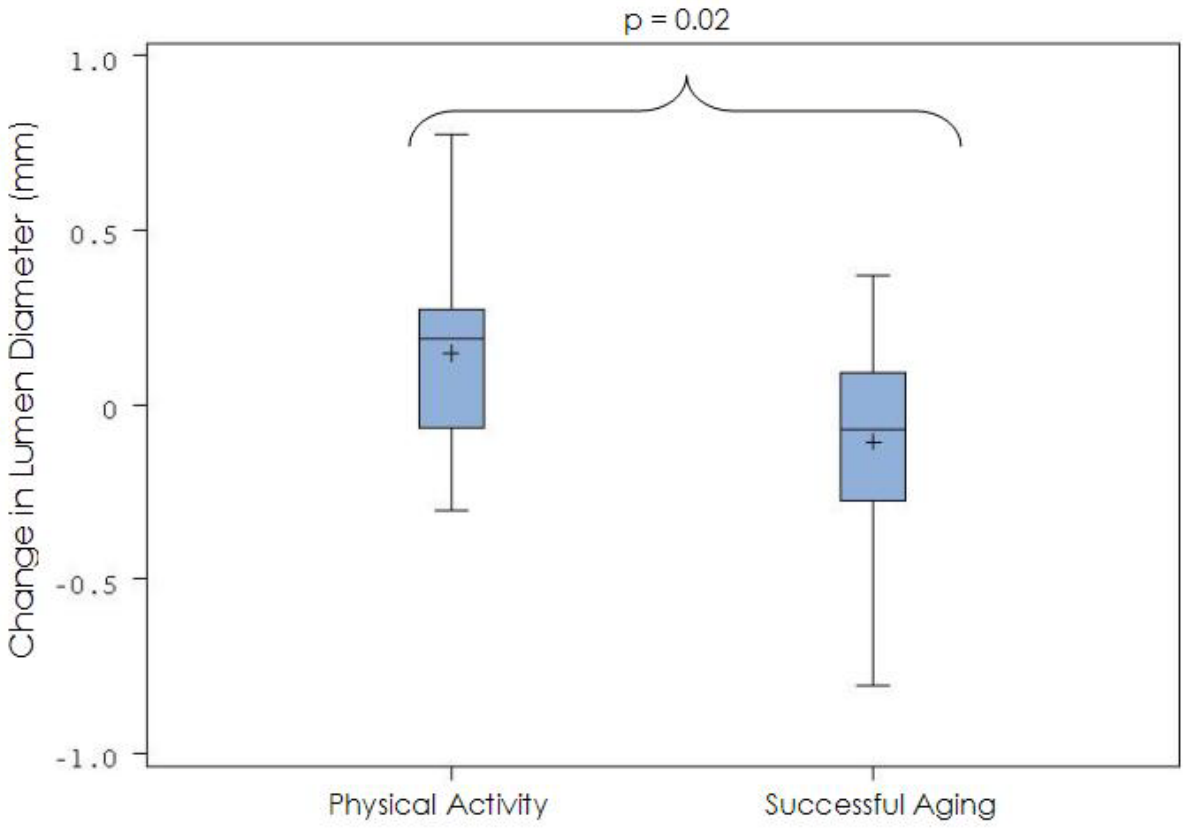


Figure 5.3 Box plot of change in lumen diameter by intervention group

P-value is from a general linear model controlling for baseline lumen diameter, race, gender, age, BMI, pulse pressure, history of hypertension and history of diabetes

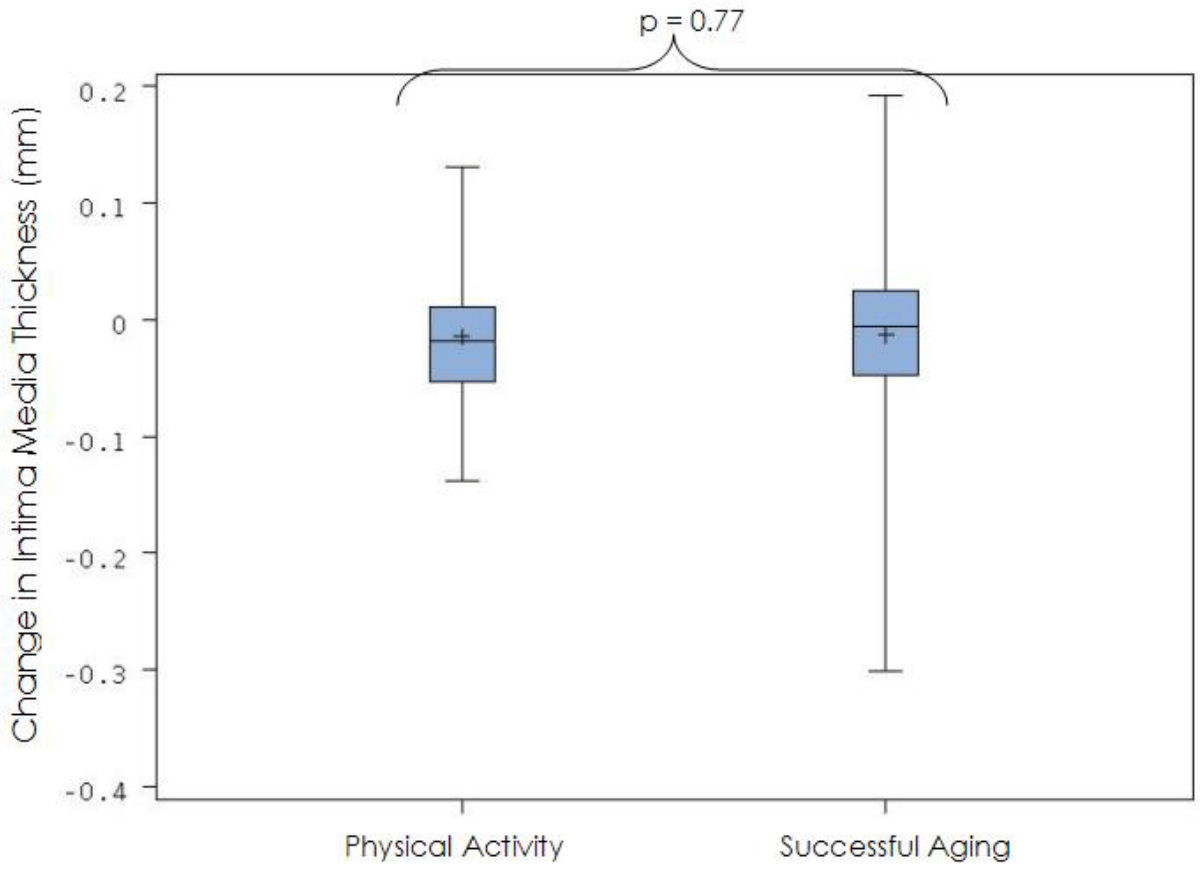


Figure 5.4 Box plot of change in intima media thickness by intervention group

P-value is from a general linear model controlling for baseline intima media thickness, race, gender, age, BMI, pulse pressure, history of hypertension and history of diabetes

# 6.0 GENERAL DISCUSSION

## 6.1 SUMMARY OF FINDINGS

This dissertation evaluated the effect of lifestyle risk factors on the vascular aging process measured by B-mode ultrasound in three cohorts that span the life course. The cohorts were from Allegheny County, Pennsylvania and the surrounding Western Pennsylvania area; the participants were overweight or obese and predominately female. All of the ultrasound measurements were made and read by trained technicians at the Ultrasound Research Lab at the University of Pittsburgh. Each study evaluated common carotid artery intima media thickness, adventitial diameter, and lumen diameter. Chapter 3 also evaluated carotid plaque. Chapters 4 and 5 included carotid-femoral pulse wave velocity.

The first cohort (Chapter 3) was composed of postmenopausal women 52-62, with a waist circumference $\geq$ 80 cm, low density lipoprotein cholesterol between 100 and 160 mg/dL, who were not diabetic, depressed, diagnosed with a

psychotic disorder or taking medication for cholesterol in the Women On the Move through Activity and Nutrition clinical trial. The second cohort (Chapter 4) was sedentary, 20-45 year old adults who were not hypertensive, diabetic or on cholesterol lowering, or vasoactive medications in the Slow Adverse Vascular Effects clinical trial. The third cohort (Chapter 5) was of sedentary, older adults 70-89 years old who were at risk for mobility disability in the Lifestyle Interventions and Independence for Elders pilot study.

Together, the papers in this dissertation, Chapters 3-5, provide evidence that lifestyle factors have an impact on vascular health. Hormone therapy users in Chapter 3, those with low risk of sleep apnea and non-snorers in Chapter 4, and older adults in a moderate physical activity intervention in Chapter 5, had significantly different vascular geometry than the hormone therapy non-users, those at high risk for sleep apnea, snorers and those in an education only control group, respectively. Each study also provides proof that the measurement of adventitial diameter is critical for having a comprehensive understanding of the vascular remodeling processes that accompany these lifestyle changes. The utility of these measures are consistent across populations at different points along the vascular aging continuum. The adaptation of the vasculature was often unseen when intima media thickness was evaluated alone; significant changes were only captured when adventitial and lumen diameter were included. Examples from Chapters 3, 4 and 5 are provided below.

Chapter 3 found current hormone therapy users had significantly smaller adventitial diameters and lumen diameters that reflect better vascular health than those who were not current users even after adjustment for cardiovascular risk factors. This study shows the importance of measuring adventitial diameter when evaluating hormone therapy; the vascular differences observed would have been overlooked if only intima media thickness had been measured. Here adventitial diameter and lumen diameter provide a more complete picture of the vascular differences between the two groups.

Men, in Chapter 4, at high risk for sleep apnea, determined by the Berlin Sleep Questionnaire[1] had significantly larger adventitial diameter and lumen diameter than those at low risk for sleep apnea; these results remained robust after adjustment for age and systolic blood pressure. Women at high risk for sleep apnea had higher intima media thickness, larger adventitial diameter and higher carotid-femoral pulse wave velocity than women at low risk for sleep apnea. After adjustment for age and systolic blood pressure the results lost significance. Compared to daytime tiredness, snoring was responsible for most of the variance observed between sleep apnea risk and subclinical cardiovascular disease. Snoring was associated with poor cardiovascular profile reflected in larger adventitial and lumen diameters in men only. These findings

provide evidence of differential vascular aging linked to sleep apnea risk and snoring in non-diabetic, normotensive, overweight and obese young adults.

In chapter 5, change in pulse wave velocity was different between the 6-month physical activity and education-only control intervention groups; with the physical activity group experiencing a clinically relevant decline in pulse wave velocity. Change in adventitial diameter and lumen diameter between groups was also different. However, contrary to expectation adventitial diameter and lumen diameter increased in the physical activity group and decreased in the control group. There were no differences in change in intima media thickness between groups. These findings reflect vascular remodeling in the adventitial and lumen diameters that is undetected when intima media thickness is evaluated. This is yet another example that highlights the value of using adventitial diameter. Despite a small sample size we were able to find differences between the groups for carotid-femoral pulse wave velocity, adventitial diameter and lumen diameter and clinically meaningful change in carotid-femoral pulse wave velocity. Further study of the effects of physical activity on subclinical cardiovascular disease in older adults is needed.

## 6.2 PUBLIC HEALTH SIGNIFICANCE

Cardiovascular disease (CVD) is the leading cause of death in the United States[2]. Although, vascular aging is part of the natural aging process, lifestyle choices can modify the trajectory of vascular aging[3]. Lifestyle choices can reverse, slow the progression or accelerate the vascular aging process thus changing the CVD risk profile. Well known traditional CVD risk factors have helped target high risk individuals. However, traditional risk factors for CVD only explain about 30% of the risk for an event. Thus additional tools that further explain CVD risk are important. CVD develops over several years beginning as fatty streaks in childhood or adolescence[4-6]. Carotid intima media thickness has become more widely used in epidemiological studies as a surrogate for atherosclerosis[7]. As such, it has been used as a tool to stratify cardiovascular risk of individuals across the subclinical disease spectrum. Although not as commonly used as intima media thickness, lumen diameter has been used with intima media thickness to provide a better picture of vascular health[8-11]. This dissertation provides confirmation that adventitial diameter should be measured with intima media thickness and lumen diameter to better target and treat those at otherwise hidden risk for cardiovascular disease. Targeting these individuals is very important because they represent a population that is

experiencing accelerated vascular aging that may not be detected through traditional risk factors alone. Identification of these individuals will allow earlier intervention that can alter the vascular aging process to prevent, slow or delay cardiovascular morbidity and / or mortality. This will also impact the burden of CVD on the healthcare system, families and the economic infrastructure. The productivity preserved by delaying morbidity by a few years or shortening the time of life spent with disability is great. This is increasingly important as the proportion of the American population over 65 years old increases rapidly with the baby boomer generation[12, 13].

The results of this dissertation have shown that the postmenopausal women with acute use of hormone therapy had smaller adventitial diameters reflecting better vascular health than those who did not use hormone therapy. This work links the effects of endogenous estrogen[14] on the vasculature with those of exogenous estrogen.

Young adult men at a high risk for sleep apnea had less favorable vascular health profiles than those with a low risk for sleep apnea. The young adults at high risk for sleep apnea reflect those on a steep trajectory to CVD events if their risks go unaddressed. It will be important to identify these young adults early so that their risk factors can be reduced with lifestyle choices or treatment. Interestingly, carotid adventitial diameter and lumen diameter in men was

different by snoring status; snorers had larger diameters than non-snorers. This work expands the literature that connects snoring and carotid artery atherosclerosis in middle aged to older men and women[15], snoring and increased CVD risk factors in men and women[16] and increased risk for CVD events in middle aged women[17].

The LIFE pilot results are important because they suggest that physical activity, even in previously sedentary older adults, provides a vascular benefit. This supports the idea that it is never too late to gain cardiovascular benefit from physical activity. Older adults in the physical activity group had significantly different change in PWV than the older adults in the successful aging control group; the regression in vascular stiffness in the physical activity group was clinically meaningful. Differences in change in AD and LD between groups were seen in opposite directions than we expected. A lesson learned from this study is that we cannot assume the effects of physical activity in older adults resemble those of younger adults. Longer duration of physical activity may be necessary to observe the expected differences in AD and LD in older adults. The results reflect the importance of studying the subclinical cardiovascular changes of older adults who participate in physical activity interventions.

## 6.3 FUTURE RESEARCH

Future research should focus on better understanding the mechanisms that explain hormone therapy, sleep apnea risk, snoring and physical activity affect the vasculature. Research to quantify the amount, the duration and who is best suited for these lifestyle choices is also needed. These questions are still being answered for hormone therapy. It is important to investigate whether lowering sleep apnea risk (i.e. snoring, obesity) translates to lowered cardiovascular risk over time in young adults. An important question is what should be done in the case of someone with chronic snoring in the absence of sleep apnea. For sleep apnea patients that snore, continuous positive airway pressure (CPAP) is the standard treatment but is not well tolerated by most patients or their bed partners[18]. A study of men with sleep apnea found that use of CPAP reduced carotid-femoral pulse wave velocity[19]; a study of the effectiveness in women should also be done. Finding a more acceptable treatment may be a key to helping this population of chronic snorers. The effect increased physical activity in older adults also needs to be further investigated. The changes in adventitial and lumen diameter indicated vascular remodeling among those in the physical activity group.

Adventitial diameter has been neglected in the literature and in common practice when carotid measurements are made. The body of research supported by this dissertation shows that adventitial diameter provides information to present a complete picture of vascular health. Adventitial diameter is very easy to measure, it can be calculated if intima media thickness and lumen diameter are available (Equation 6.1). The common practice of digitally recording carotid ultrasonography would allow re-reading the images to measure adventitial diameter if intima media thickness and lumen diameter were not both measured. Prior studies of lifestyle factors that used carotid measures should evaluate the results when adventitial diameter is included. This is especially important in studies that had null findings because intima media thickness cannot capture the subtle changes in the adventitia. Thus, future studies of lifestyle interventions that use carotid measures as the outcome should include adventitial diameter.

**Equation 6.1** To calculate adventitial diameter from lumen diameter and intima media thickness

$$\text{Adventitial diameter} = \text{lumen diameter} + \text{intima media thickness} \times 2$$

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